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UNIVERSITY OF ALBERTA

APPLICATION OF ULTRASOUND TO DISSOLUTION OF BIOLOGICAL MATERIALS FOR ELEMENTAL ANALYSIS

by

Sipho Mduduzi Mamba ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of **Doctor of Philosophy**.

DEPARTMENT OF CHEMISTRY

Edmonton, Alberta

Spring, 1995

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S.M. Mamba.

Sipho Mduduzi Mamba

Permanent Address: Department of Chemistry
University of Swaziland
Kwaluseni Main Campus
Kingdom of Swaziland, Africa

Date: 23 February 1995

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled:

Application of Ultrasound to Dissolution of Biological Materials for Elemental Analysis

submitted by **Sipho Mduduzi Mamba** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy**.

B. Kratochvil

B. Kratochvil, Supervisor

J. A. Plambeck

N. J. Dovichi

A. H. Kalantar

M. J. Dudas

E. S. Salin, External Examiner

Date: 23 Feb 95

To My Family

Assienah, James, Jabulane, Sbusiso, Kurrah, Nhlanhla and Wandile

My Special Friends

Roberta Michele, Jenifer Benoit and Gwen Allore

and My Buddies

Steve Phakisi, George Ndana, Enoch Dlamini, Scud, Wes Allore, Marc Rivet, Sarah MacDougal, Mbangi Nepfumbada, Alebachew Demoz, Portia Makola and Kingsley Donkor.

Abstract

The application of ultrasonic energy to the dissolution of biological materials for elemental chemical analysis is described. The method ultimately developed employs a commercial cell disruptor unit operating at a fixed frequency of 20 kHz and at power up to 600 W. A titanium probe is immersed in a suspension of the sample in a solution containing dilute sulfuric acid and hydrogen peroxide.

In the presence of dissolved gases, sonication at 20 kHz and powers of several hundred watts causes acoustic cavitation, wherein the rapid formation and collapse of gas bubbles generate temperatures of up to 5000 °C and pressures of up to 1000 atm. Cavitation acts on suspended material in two ways. First, it mechanically breaks up the solid to expose a larger surface area to the solvents used for dissolution (sonomechanical effect). Second, it produces oxidants, such as OH radicals and hydrogen peroxide from water, that attack sample particles (sonochemical effect). Both processes increase rates of dissolution. Furthermore, the generation of oxidizing species *in situ* reduces the amounts of external reagents that must be added, and thereby reduces the likelihood of contamination from reagents.

The physical effects of sonication on a range of materials, including chalk, Cu wire, Al foil, bovine hemin, dried bovine liver and granular cellulose, were investigated by optical and scanning electron microscopies. Disintegration was marked for both inorganic and organic materials. The least affected was the relatively fatty liver tissue.

The chemical effects of sonication were studied in two stages. In the first stage the production of oxidizing species as a function of dissolved gas, ultrasound power, temperature, and presence of solutes H^+, SO_4^{2-}, Cl^-, F^-, and NO_3^- was measured by oxidation of iodide to iodine. In the second stage a range of

conditions were investigated for the dissolution of several biological reference materials. The optimum solvent composition was found to be 2.5% H_2O_2 in 0.5 M H_2SO_4. With this solvent system quantitative dissolution was achieved for several reference materials, including lobster, mussel and oyster tissue, rice flour, pine needles, citrus leaves, bovine liver, and corn stalk, as well as human hair, in about 40 min for 250-mg test portions as shown by analyses using inductively coupled plasma-atomic emission spectroscopy.

The commercial titanium alloy sonication probe used in this study introduced contamination by several metals, particularly aluminum and vanadium, into the sample solutions. Several modifications to the probe were studied in attempts to alleviate the problem, including covering with a Teflon sleeve, replacing the titanium alloy tip with one of 99.97% pure titanium, and replacing the lower half of the probe with a rod of pure titanium. The best solution was to replace the removable tip with an extender rod of pure titanium. This lowered contamination to negligible levels for all but iron. The extended probe had the disadvantage, however, that it could not be operated longer than 10 minutes at a time because of heat build-up at the extender-probe joint.

Overall, the use of ultrasonic energy to speed up sample dissolution for elemental chemical analysis was shown to be a highly promising method that is worth further study.

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TABLE OF CONTENTS

CHAPTER PAGE

1. Introduction.....1

1.1 Elemental Analysis: An Overview.....1

1.1.1 Elemental Analysis in the Clinical Sciences.....1

1.1.2 Elemental Analysis in Environmental Science.....2

1.1.3 Elemental Analysis in Food and Agricultural Sciences.....3

1.1.4 Elemental Analysis and Biomonitoring Programs.....3

1.1.5 Productivity in the Elemental Analysis Laboratory: Present and Future Demand.....3

1.2 A Survey of Techniques Used For Dissolution of Solid Samples.....4

1.2.1 The Role of Sample Dissolution in Elemental Analysis.....5

1.2.2 Traditional Methods of Dissolution.....5

1.2.2.1 Thermal Open Vessel Decomposition: Dry Ashing.....5

1.2.2.2 Open-Vessel Chemical Digestion: The "Hot-Plate" Method.....5

1.2.3 Modern Methods of Dissolution.....8

1.2.3.1 Hot-Block Digestors.....8

1.2.3.2 Closed-Vessel Acid Digestion.....10

1.2.3.3 Closed Vessel Microwave Digestion.....11

1.3 Alternative Approaches to The Problem of Sample Dissolution for Elemental Analysis.....14

1.3.1 Background 14

1.3.2 Avoiding Dissolution by the Direct Analysis of Solids.....14

1.3.2.1 Slurry Techniques.....15

1.3.2.2 Solid Sample Insertion Methods.....15

1.3.2.3 Current Problems with Direct Analysis of Solids....16
1.3.3 Summary of Trends in Sample Preparation Research....17
1.3.4 Background for Consideration of an Ultrasound-Based Dissolution System....18
1.4 Ultrasound: A General Overview....18
1.4.1 Properties of Ultrasound Waves....19
1.4.2 Generation of Ultrasonic Waves....20
1.4.3 Ultrasound Applications in Industry, Biology and Medicine....21
1.4.4 Ultrasound Applications in Chemistry....22
1.4.4.1 Ultrasound Synthesis of Organic Compounds....22
1.4.4.2 Ultrasound Synthesis of Inorganic Compounds....23
1.4.4.3 Ultrasound Polymer Degradation....24
1.4.4.4 Applications of Ultrasound in Analytical Chemistry....24
1.4.4.4.1 Ultrasonic Nebulization and Slurry Stabilization....24
1.4.4.4.2 Ultrasonic Extraction of Metals....25
1.5 Summary and Goals of this Study....27
1.5.1 Summary....27
1.5.2 Aims and Objectives....27
2. Study of Processes that Occur on Application of Ultrasound Energy to Two-Phase Liquid-Solid Systems....29
2.1 Introduction....29
2.1.1 Acoustic Cavitation....29
2.1.2 Sources of High Intensity Ultrasound in the Laboratory....31
2.1.3 Aims and Objectives of this Study....32
2.2 Experimental Section....33

2.2.1 Equipment....33
2.2.1.1 Ultrasound Unit....33
2.2.1.2 Optical Microscopy....35
2.2.1.3 Scanning Electron Microscopy (SEM)....35
2.2.2 Reagents and Materials....35
2.2.3 Methods....36
2.2.3.1 Sonication Procedure....36
2.2.3.2 Preparation of Samples for SEM Studies....36
2.2.3.3 Iodimetric Determination of Copper....36
2.3 Results and Discussion....37
2.3.1 Evidence of Cavitation: Sonication of Chalk and Al Foil....37
2.3.1.1 Sonication of Chalk....37
2.3.1.2 Sonication of Al Foil....39
2.3.2 Cavitation in Relation to Ultrasound Dissolution....43
2.3.3 Effect of Ultrasound Cavitation on Titanium and Copper Metal....43
2.3.3.1 SEM Study of Erosion of a Commercial Titanium Alloy Horn Tip....45
2.3.3.2 Experiments with Copper Wire....45
2.3.3.3 Possible Mechanisms of Cavitational Damage....50
2.3.4 Morphological Studies of Insonated Biological Sample Surfaces....50
2.3.4.1 Morphological Studies of Bovine Hemin....51
2.3.4.2 Morphological Studies of a Dried Bovine Liver Reference Material....54
2.3.4.3 Morphological Studies of Granular Cellulose....54
2.4 Summary....60

3. Study of Processes that Occur on Application of Ultrasound to Homogeneous Single Phase Aqueous Systems 61

3.1 Introduction 61

3.1.1 A Brief Review of Chemical Reagents Generated by Ultrasound 62

3.1.2 Acoustic Cavitation 62

3.2 Experimental 64

3.2.1 Reagents and Solutions 64

3.2.2 Equipment 64

3.2.2.1 Sonication Apparatus 64

3.2.2.2 Uv-visible Spectrophotometry of Hydrogen Peroxide 64

3.2.3 Methods 66

3.2.3.1 Determination of H_2O_2 66

3.2.3.1.1 Iodometric Titration 66

3.2.3.1.2 Spectrophotometric Measurement 66

3.2.3.1.3 Modification of Kingzet Method for Peroxide Determination 67

3.2.3.2 Polarographic Determination of O_2 74

3.3 Results and Discussion 74

3.3.1 Effect of Sonication Time on Ultrasound-Produced Hydrogen Peroxide 76

3.3.2 Effect of Probe Location in the Sonication Vessel 78

3.3.3 Effect of Ultrasound Power 80

3.3.4 Effect of Cavitation Gas on Generation of Oxidizing Species 82

3.3.4.1 Cavitation in the Presence/Absence of Dissolved Gases 82

3.3.4.2 A Sonochemical Model For Gaseous Cavitation 83

3.3.4.3 Evaluation of Nitrogen and Argon as Cavitation Gases 88

3.3.4.4 Evaluation of Oxygen as Cavitation Gas 88

3.3.4.4.1 Measurement of Dissolved Oxygen.....90
3.3.4.4.2 The Effect of Oxygen on Formation of Oxidizing Species.....91
3.3.4.4.3 Effect of Oxygen Levels During Sonication.....96
3.3.5 Effect of Temperature on Production of Oxidizing Species.....99
3.3.5.1 Measurement of Temperature During Sonication 99
3.3.5.2 Effect of Temperature on Production of Oxidants by Sonication....100
3.3.5.3 Effect of Temperature on the Stability of Solutions of Peroxide....102
3.3.5.4 Summary of Temperature Studies.....105
3.3.6 Effect of Acid on H_2O_2 Production.....105
3.3.6.1 Sonication in Dilute H_2SO_4.....105
3.3.6.2 Sonication in Dilute HNO_3.....106
3.3.6.3 Sonication in Dilute HCl.....108
3.3.6.4 Sonication in Dilute HF.....111
3.3.6.5 Sonication in Mixtures of Acids.....111
3.4 Conclusions.....112
4. Studies of Effect of Solvent Composition on Dissolution of Biological Materials: Ultrasound versus Heat.....115
4.1 Introduction.....115
4.1.1 Biological Sample Dissolution: Ultrasound vs Heat Energy.....115
4.1.2 Method Development.....116
4.1.2.1 Use of Model Compounds and Certified Reference Materials.....116
4.1.2.2 Multi-element Determinations Using the ICP as an Excitation Source.....119
4.2 Experimental.....120
4.2.1 Materials.....120
4.2.2 Solutions.....121

4.2.3 Sonication Procedure....121
4.2.4 ICP Atomic Emission Measurements....121
4.3 Results and Discussion....122
4.3.1 Study of the Effect of Ultrasound on the Dissolution of $Ni(DMG)_2$....122
4.3.1.1 Calibration of the ICP-OES for Ni....122
4.3.1.2 Study of Possible Ni Contamination from Ultrasonic Probe....123
4.3.1.3 Sonication of $Ni(DMG)_2$ in $H_2O/CHCl_3$ Mixtures....123
4.3.1.4 Sonication of $Ni(DMG)_2$ in 0.5 M H_2SO_4....126
4.3.1.5 Sonication of $Ni(DMG)_2$ in Water and 0.5% H_2O_2....128
4.3.1.6 Summary of $Ni(DMG)_2$ Results....131
4.3.2 Comparison of Ultrasound and Heat for the Decomposition of Hemin.131
4.3.2.1 ICP-OES Calibration for Fe....131
4.3.2.2 Effect of Concentrated Acid on Hemin....133
4.3.2.3 Effect of Heat and Ultrasound on Hemin in Water....135
4.3.2.4 Effect of Heat and Ultrasound on Hemin in Dilute Acid....135
4.3.2.5 Effect of Heat and Ultrasound on Hemin in H_2O_2....140
4.3.2.6 Effect of Heat and Ultrasound on Hemin in Acidic Solutions of Hydrogen Peroxide....141
4.3.2.7 Summary of Results of Hemin Study....145
4.3.3 Application of H_2O_2/H_2SO_4 Mixtures as Solvent for the Decomposition of Biological Samples by Sonication....145
4.3.3.1 Procedure and Results for TORT-1, Lobster Tissue CRM....146
4.3.3.2 Procedure and Results for NIST 1638a, a Rice Flour SRM....152
4.3.3.3 Summary of Results Obtained for TORT-1 and Rice Flour....154
4.4 Summary....156

5. Application of Ultrasound to Dissolution of Biological Materials for Elemental Analysis by ICP-OES.....157

5.1 Introduction.....157

5.1.1 General.....157

5.1.2 Evaluation of Performance of Ultrasound Dissolution Scheme.....158

5.2 Experimental.....158

5.2.1 Materials.....158

5.2.2 Solutions.....159

5.2.3 Sonication Procedure.....159

5.2.4 ICP Atomic Emission Measurements.....160

5.3 Results and Discussion.....160

5.3.1 Application to Environmental Samples.....161

5.3.1.1 Dissolution of Powdered Mussel Tissue and the Effect of Sample Weight.....161

5.3.1.2 Dissolution of Powdered Lobster Tissue.....170

5.3.1.3 Dissolution of Pine Needles.....172

5.3.1.4 Dissolution of High-Cadmium Unpolished Rice Flour.....174

5.3.2 Application to Agricultural Samples.....176

5.3.2.1 Dissolution of Powdered Citrus Leaves.....176

5.3.2.2 Dissolution of NIST Rice Flour.....178

5.2.3.3 Dissolution of Agriculture Canada Corn Stalk Reference Material.178

5.3.3 Application to Food Samples.....181

5.3.3.1 Dissolution of Oyster Tissue.....181

5.3.3.2 Dissolution of Whole Milk Powder.....181

5.3.4 Application to Clinical Samples.....184

5.3.4.1 Dissolution of Acetone-Extracted Bovine Liver, NIST SRM 1577a....184
5.3.4.2 Dissolution of Human Hair....186
5.4 Summary....188
6. Contamination Assessment and Design of Horns for Ultrasound Dissolution of Biological Materials....189
6.1 General....189
6.2 Contamination Assessment from a Commercial Ultrasonic Probe....189
6.2.1 Introduction....189
6.2.2 Principles of INAA....190
6.2.3 Experimental....191
6.2.3.1 Materials and Reagents....191
6.2.3.2 Sonication Apparatus....191
6.2.3.3 Microwave Evaporation Apparatus....191
6.2.3.4 University of Alberta SLOWPOKE-2 Nuclear Reactor Facility....191
6.2.3.5 Methods....193
6.2.3.5.1 Preparation of Blank Solutions for INAA....193
6.2.3.5.2 INAA Procedure for Elemental Analysis of Blank Solutions....193
6.2.3.5.3 INAA Procedure for Elemental Analysis of Commercial Ti Probe Tip....194
6.2.3.5.4 Elemental Analysis of Solutions Using ICP-OES....194
6.2.4 Results and Discussion....194
6.2.4.1 Extent of Erosion of Ti Probe Tip....194
6.2.4.2 INAA Studies of Sonicated Solutions....195
6.2.4.3 ICP-OES Studies of Sonicated Solutions....197
6.2.4.4 INAA Studies and HF Etching Experiments on Titanium Probe....199

6.2.5 Summary of Results on Commercial Probe.....202
6.3 Modification of Horns for Ultrasound Dissolution.....202
6.3.1 Introduction.....202
6.3.2 Evaluation of a Teflon Sleeve Cover for Ultrasound Probes.....203
6.3.2.1 Experimental.....203
6.3.2.2 Results and Discussion.....205
6.3.2.3 Summary.....206
6.3.3 Design and Evaluation of Horn Components Made of Pure Titanium..206
6.3.3.1 Fabrication and Evaluation of Performance of Pure Titanium Tips.....206
6.3.3.1.1 INAA Study of Trace Elemental Concentrations in High Purity Ti Rods.....208
6.3.3.1.2 ICP-OES Analysis of Blanks from Sonication with a Pure Ti Probe Tip.....208
6.3.3.1.3 Conclusions.....211
6.3.3.2 Fabrication of Pure Titanium Rods for Ultrasonic Dissolution.....211
6.3.3.2.1 General: The Crevice as a Source of Contamination.....211
6.3.3.2.2 Fabrication of Pure Ti Rods.....212
6.3.3.2.3 Tuning of Modified Probe.....214
6.3.3.2.4 Conclusions.....215
6.3.3.3 Fabrication and Evaluation of Perfomance of Pure Ti Extenders215
6.3.3.3.1 Analysis of Pure Ti Extender Blanks by ICP-OES.....215
6.3.3.3.2 Application to Elemental Analysis of Human Hair.....216
6.4 Summary of Contamination Study.....219
7. Summary of Results and Suggestions for Further Work.....221
7.1 Summary.....221

7.2 Suggestions for Further Work.....226
7.2.1 Identification of Chemical Species Produced by Cavitation.....226
7.2.2 Application to Materials Other Than Biological Samples.....227
7.2.3 Kinetic Studies.....228
7.2.4 Ultrasound Equipment.....228
References.....230

LIST OF TABLES

Table		Page
1.1	Classification of Sound Waves	18
2.1	Effect of Sonication on Aluminum Foil	42
2.2	Effect of Sonication on Copper Wire	49
3.1	Operating Conditions for the PAR 174A Polarographic Analyzer	75
3.2	Summary of Thermal Properties of Several Laboratory Gases	87
3.3	Oxygen and Oxidants (as Peroxide) Concentration in Unsonicated Water Samples as a Function of Equilibration with Air or O_2 Gas.	
3.4	Effect of Heating on the Concentration of Hydrogen Peroxide Remaining in a Solution Having an Initial Concentration of 0.020 mM	104
4.1	Effect of Sonication and Addition of Acid on ICP-OES Signal Intensity for Nickel	125
4.2	Total Amount of Ni Found in Aqueous Phase After Sonication of $Ni(DMG)_2$ in $CHCl_3/H_2O$ Mixtures	127
4.3	Release of Iron from Hemin in Concentrated Mineral Acids	134
4.4	Comparison of Experimental Results with Certified Values for Three Elements in NIST SRM 1638a, Rice Flour	155
5.1	Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values for NIES #6, Powdered Mussels	162
5.2	Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values for TORT-1, Lobster Hepatopancreas	171
5.3	Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values for SRM #1575, Pine Needles	173

5.4 Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values for NIES #10c, High-Cadmium Unpolished Rice Flour.....175

5.5 Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values for SRM #1572, Citrus Leaves.....177

5.6 Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values for SRM #1568a, Rice Flour.....179

5.7 Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values for Agriculture Canada Reference Material 8412, Corn (*Zea mays*) Stalk.....180

5.8 Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values for SRM #1566, Oyster Tissue.....182

5.9 Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values for Agriculture Canada RM 8435, Whole Milk Powder.....183

5.10 Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values for SRM #1577a, Bovine Liver..... 185

5.11 Elemental Concentrations in Sonicated Human Hair Samples Measured by ICP-OES.....187

6.1 INAA Results for Ti, Al and V in Sonicated Solutions.....196

6.2 ICP-OES Results for 0.5% H_2O_2/0.5 M H_2SO_4 Solutions Sonicated for Varying Times Using a 0.5" Diameter Sonics Probe.....198

6.3 INAA Results for a Sonics Commercial Ti Tip.....201

6.4 INAA Results for Pure Ti Metal Used to Fabricate Tips, Rods and Extenders for Ultrasonic Probes.....209

6.5 ICP-OES Results for 0.5% H_2O_2/0.5 M H_2SO_4 Solutions as a Function of Sonication Time Using a 0.5" Diameter Sonics Probe After Replacement of the Ti Alloy Tip with one Fabricated from Pure Ti.....210

6.6 Results of ICP-OES Analyses for Elements Found in Crevice Between Probe and Ti Alloy Tip.....213

6.7 Results of ICP-OES Analyses for 0.5% H_2O_2/0.5 M H_2SO_4 Solutions Sonicated for Varying Times Using a 0.5" Diameter Sonics Probe in which the Ti Alloy Tip was Replaced with an Extender Fabricated From Pure Ti 217

6.8 Results for Analysis of Human Hair.....218

LIST OF FIGURES

Figure		Page
1.1	Overall Scheme for Elemental Analysis	6
1.2	Time Taken to Complete Sample Dissolution in Relation to Total Analysis Time	9
1.3	Source of Errors in Sample Analysis	9
2.1	Ace Glass 9810 Assembly Used to Study Processes that Occur on Application of 20 kHz Ultrasound Energy to Two-Phase Solid-Liquid Systems	34
2.2	(i) Photomicrograph of Chalk Taken Before Irradiation with Ultrasound	38
2.2	(ii) Photomicrograph of Chalk Taken After Irradiation with Ultrasound for 1sec	38
2.2	(iii) Photomicrograph of Chalk taken After Irradiation with Ultrasound for 5 sec	40
2.2	(iv) Photomicrograph of Chalk Taken After Boiling in Water for 1 hr	40
2.3	(i) Photomicrograph of Al Foil Taken Before Irradiation with Ultrasound	41
2.3	(ii) Photomicrograph of Al Foil Taken After Irradiation with Ultrasound for 5 sec	41
2.3	(iii) Photomicrograph of Al foil Taken After Irradiation with Ultrasound for 20 sec	44
2.3	(iv) Photomicrograph of Al Foil Taken After Irradiation with Ultrasound for 120 sec	44
2.4	(i) SEM Photomicrograph of a New Ti Alloy Horn Tip	46
2.4	(ii) SEM Photomicrograph of a Ti Alloy Horn Tip After 15 hrs of Use	47

2.4 (iii) SEM Photomicrograph of a Ti Alloy Horn Tip After 15 hrs of Use and Polishing with Fine Emery Cloth....48
2.5 (i) SEM Photomicrograph of a Hemin Particle Before Sonication....52
2.5 (ii) SEM Photomicrograph of a Hemin Particle After Sonication in Water for 30 min....52
2.5 (iii) SEM Photomicrograph of a Hemin Particle After Sonication in Water for 1 hr....53
2.5 (iv) SEM Photomicrograph of a Hemin Particle After Boiling in Water for 1 hr....53
2.6 (i) SEM Photomicrograph of Bovine Liver Powder Before Sonication....55
2.6 (ii) SEM Photomicrograph of Bovine Liver Powder After Sonication in Water for 30 min....55
2.6 (iii) SEM Photomicrograph of Bovine Liver Powder After Sonication in Water for 1 hr....56
2.6 (iv) SEM Photomicrograph of Bovine Liver Powder After Boiling in Water for 1 hr....56
2.7 (i) SEM Photomicrograph of Granular Cellulose Before Sonication.... .58
2.7 (ii) SEM Photomicrograph of Granular Cellulose After Sonication in Water for 30 min....58
2.7 (iii) SEM Photomicrograph of Granular Cellulose After Sonication in Water for 1 hr....59
2.7 (iv) SEM Photomicrograph of Granular Cellulose After Boiling in Water for 1 hr....59
3.1 Schematic Diagram of a Sonochemical Reaction Assembly for the Study of Sonication Processes in a Homogeneous Single-Phase Aqueous System...65

3.2 Absorption Spectrum of I_3^- Ion....68
3.3 Effect of Ammonium Molybdate Catalyst on the Rate of Peroxide-Iodide Reaction....69
3.4 Absorbance as a Function of Wavelength for I_3^- Produced from Hydrogen Peroxide Standards....71
3.5 Calibration Plot for Determination of Hydrogen Peroxide....72
3.6 Absorption Spectrum of a Water Sample (after development of color) that was Sonicated for 30 min....73
3.7 Levels of Oxidant Produced (as hydrogen peroxide) from Water in Equilibrium with Air as a Function of Sonication Time....77
3.8 Effect of Position of Probe Tip Relative to Sonication Vessel Bottom, on Oxidant Levels in Sonicated Water....79
3.9 Effect of Ultrasound Power on Oxidant levels in Sonicated Water Samples, as Measured by Iodide Conversion to I_3^-....81
3.10 Effect of Presence or Absence of Gas on the Sonochemical Yield of Hydrogen Peroxide....84
3.11 Effect of Cavitation Gas Composition on Sonochemical Yields of Oxidants, Reported as Hydrogen Peroxide....89
3.12 Effect of Dissolved Oxygen Concentration on Formation of Oxidizing Species, Measured as Hydrogen Peroxide, in Sonicated Water....92
3.13 Effect of Volume on the Concentration of Oxidants (measured as peroxide) Formed in Water Initially in Equilibrium with Air as a Function of Sonication Time at 600 W....95
3.14 Dissolved Oxygen Concentration in Water as a Function of Sonication Time Under Various Levels of Oxygen Exposure....97

3.15 Effect of Sonication Time on the Temperature of an 80-mL Sample of Water....101
3.16 Effect of Partial Temperature Control on the Concentration of Oxidants (measured as peroxide) Formed in 80-mL Portions of Water, Initially in Equilibrium with Air, as a Function of Sonication Time at 600 W..... ..103
3.17 Effect of H_2SO_4 and Na_2SO_4 Concentration on the Formation of Oxidising Species....107
3.18 Effect of HNO_3 and $NaNO_3$ Concentration on the Formation of Oxidising Species....109
3.19 Effect of HCl and NaCl Concentration on the Formation of Oxidising Species....110
4.1 ICP Calibration Curve for Nickel....124
4.2 Quantity of Free Nickel Released Upon Sonication or Boiling for 30 minutes of 50 mL of Solution Containing 2 mg of Nickel Initially as $Ni(DMG)_2$....130
4.3 ICP Calibration Curve for Fe....132
4.4 Effect of Heat and Sonication on Fraction of Iron Released from Hemin in Water as a Function of Sonication Time....136
4.5 Effect of Heat and Ultrasound Treatment on Dissolution of Hemin as a Function of Time in Dilute Sulfuric Acid.... ...137
4.6 Effect of Heat and Ultrasound Treatment on Dissolution of Hemin as a Function of Time in Dilute Hydrochloric Acid....138
4.7 Effect of Heat and Ultrasound Treatment on Dissolution of Hemin as a Function of Time in Dilute Nitric Acid....139

4.8 Effect of Heat and Ultrasound on Release of Fe from Hemin into Solution at Various Concentrations of H_2O_2.....142
4.9 Effect of Heat and Ultrasound on Release of Fe from Hemin into Solution at Various Concentrations of H_2O_2 in 0.5 M H_2SO_4.....144
4.10 Amount of Dissolved Cu Found in Sonicated Solutions of TORT-1 as a Function of Sonication Time.....147
4.11 Amount of Dissolved Cd Found in Sonicated Solutions of TORT-1 as a Function of Sonication Time.....148
4.12 Amount of Dissolved Mn Found in Sonicated Solutions of TORT-1 as a Function of Sonication Time.....149
4.13 Amount of Dissolved Sr Found in Sonicated Solutions of TORT-1 as a Function of Sonication Time.....150
4.14 Amount of Dissolved Fe Found in Sonicated Solutions of TORT-1 as a Function of Sonication Time.....151
4.15 Amount of Dissolved Cu Released into Solution During Sonication of TORT-1 in 0.5 M H_2SO_4 as a Function of Sonication Time and Concentration of H_2O_2.....153
5.1 ICP-OES Emission Signals for Cu in Several Reference Materials.....164
5.2 ICP-OES Emission Signals for Cd in Several Reference Materials.....165
5.3 ICP-OES Emission Signals for Zn in Several Reference Materials.....166
5.4 ICP-OES Emission Signals for Sr in Several Reference Materials.....167
5.5 ICP-OES Emission Signals for Mn in Several Reference Materials.....168
5.6 ICP-OES Emission Signals for Fe in Several Reference Materials.....169
6.1 Amounts of As, V and Al Appearing in Sonicated Solutions of 0.5% H_2O_2 in 0.5 M H_2SO_4 as a Function of Sonication Time.....200
6.2 Schematic Diagram of a Teflon-Sleeved Commercial Sonics Probe.....204
6.3 Design of Ultrasound Horns for Dissolutions.....207

CHAPTER I

INTRODUCTION

1.1 Elemental Analysis: An Overview

Information about elemental concentrations in biological materials is important because of their importance for plant and animal development. Although some are essential in varying amounts, many others become toxic above threshold concentrations.

1.1.1 Elemental Analysis in the Clinical Sciences

The analysis of elements in body fluids and tissues allows us to check the levels of essential mineral nutrients that play a major role in health and disease. Iron is required for the synthesis of hemoglobin, for example, and its deficiency leads to chronic fatigue as a result of reduced capacity of the blood to carry oxygen. Copper is essential for the synthesis of a variety of metalloenzymes, and its deficiency results in nutritional anemia in children. Trace amounts of cobalt are required for the synthesis of vitamin B_{12}, and a deficiency leads to impairment of liver functions. Zinc is essential for retinol synthesis; its deficiency leads to blindness. Selenium is required for the synthesis of selenocysteine, selenomethionine and glutathione peroxidase enzyme. Excess quantities of these metals, however, often result in adverse physiological disorders, ranging from heart disease to kidney failure.[1,2]

1.1.2 Elemental Analysis in Environmental Science

Concerns about the deteriorating quality of the environment have led to an increased demand for quantitative information about elements such as Pb, Cd and As in biological materials. These comprise a group of elements that have no known function in humans, but often give rise to adverse physiological disorders at levels as low as a part-per-billion (ppb). These elements are present in industrial and domestic waste.[3] Cadmium, a well-known neurotoxin[4], causes "Itai-Itai" (Ouch-Ouch), a disease prevalent among Japanese who consume rice and drinking water contaminated with it.[5] Blood levels of lead greater than 10 ppb cause learning and behavior disorders in children, impair central nervous system development in fetuses, and raise the blood pressure of pregnant women.[6] Elevated blood levels in men leads to muscle pain, fatigue and abdominal colic.[7] Even though regulatory steps have been taken by government agencies,[8,9] such as the US Center for Disease Control and Prevention, to reduce exposure to lead, little or no effort is being made by developing countries towards this goal.[10] In these countries, domestic water distribution systems may still use lead pipes, all gasoline supplies contain tetraethyl lead antiknock additives[11], children are regularly exposed to peeling paint[12] and improperly discarded lead acid batteries.

Other trace metals that are considered industrial environmental pollutants include strontium, barium, tin, silver and mercury.[13-15] Some essential elements, such as chromium, cobalt, molybdenum, copper, iron, zinc, nickel, manganese and selenium, are toxic at high concentrations, and are considered industrial environmental pollutants at elevated levels.

1.1.3 Elemental Analysis in Food and Agricultural Sciences

Analysis of plant tissues for trace elements is routinely performed to assess the mineral nutritional value of agricultural products, the main source of essential elements required for healthy growth and development. In human metabolic balance studies, foods, diet composites and fecal samples are routinely analyzed for a variety of essential elements.[16] Since some of the feeds used in the commercial production of livestock, and some soils used to raise food crops, are exposed to sludge material, contamination by heavy metals is a real possibility.[17] Metal pollutants are routinely determined at trace levels in meat, fish, fruit, vegetables, cereals, wines, and milk.[18-20]

1.1.4 Elemental Analysis and Biomonitoring Programs

Elemental analysis of biological materials also helps us to assess the levels of pollutants in the environment. In the 1970's, The National Institute of Standards and Technology established a National Biomonitoring Specimen Bank to establish trace element reference values in human tissue.[21,22] Several countries worldwide have embarked on similar projects. For example, the European Community routinely analyses human blood for trace metals in order to establish baseline data for trace elements in human body fluids.[23] They also analyze aquatic life forms and foliage[24,25] on a regular basis.

1.1.5 Productivity in the Elemental Analysis Laboratory: Present and Future Demands

As the demand for monitoring of elements in a wide range of materials continues to grow, the analytical laboratory of the 1990's is under tremendous pressure to meet the challenges placed on it by the diversity and sheer numbers of samples that have to be processed. In 1986, Worthy *et al.* reported that

approximately 250 million analyses are conducted daily in the U.S., and carry an annual price tag of $50 billion[26]. These numbers have undoubtedly doubled, or even tripled, over the past decade.

In order to meet the demands of the 1990's, increased productivity in the analytical laboratory is required.[27] As well, methods of analysis need to be improved in terms of economy and speed.[28,29] With the advent of microprocessor technology in analytical instrumentation, accuracy, precision and speed of analyses have greatly improved.[30,31] Today, a majority of analytical instruments are fully automated, can be run overnight (unattended operation), and use so called "intelligent software systems" with in-built feedback mechanisms. The result is increased sample throughput and a corresponding sharp reduction of costs associated with labor.[32]

Unfortunately, most of the developments mentioned here deal with the data acquisition and manipulation end of chemical analysis. Analytical chemists have not spent much effort on improving the steps prior to measurement, such as sample dissolution. In this thesis, a new technique for dissolving biological samples is introduced. The method is intended to complement rather than replace traditional methods, which have changed little in the past several decades.

1.2 A Survey of Techniques Used For Dissolution of Solid Samples

A number of methods have been developed over the years to determine metals in biological materials at part per million (ppm) levels and lower. Among the most successful and widely used are inductively coupled plasma atomic emission spectroscopy (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), atomic absorption spectroscopy (AAS) and anodic stripping voltammetry (ASV).[33] All require the sample to be dissolved prior to measurement.

1.2.1 The Role of Sample Dissolution in Elemental Analysis

Figure 1.1 shows the various steps involved when a sample is brought to the laboratory for analysis using the instrumental techniques listed above. For solids, these techniques require that the solid sample be dissolved, without analyte loss,[34] prior to measurement of the resulting solutions. Samples may contain a variety of organic and inorganic compounds, including refractory minerals such as zircon and aluminosilicates.[35-37] In a majority of biological materials, the elements of interest are usually bound to an organic matrix, which must be decomposed prior to measurement.[38]

1.2.2 Traditional Methods of Dissolution

Several methods for dissolution of samples prior to analysis have been developed over the past decade or so.[39-42] Commonly used procedures for decomposition of organic matter in biological materials include dry ashing and chemical digestion using oxidizing reagents. These are discussed below.

1.2.2.1 Thermal Open Vessel Decomposition: Dry Ashing

Dry ashing involves the high temperature combustion of organic matter in a muffle furnace and subsequent dissolution of the mineral constituents, usually in acid.[43] The procedure is time consuming, and is further complicated by the possibility of volatilization losses during the combustion stage, as well as difficulty in completely dissolving the ash.[44-46]

1.2.2.2 Open-Vessel Chemical Digestion: The "Hot-Plate" Method

Over the past four decades or so, wet ashing has undoubtedly become the most popular digestion method, since analyte loss through volatilization occurs to a smaller extent when compared to dry ashing procedures.[41,47,48]

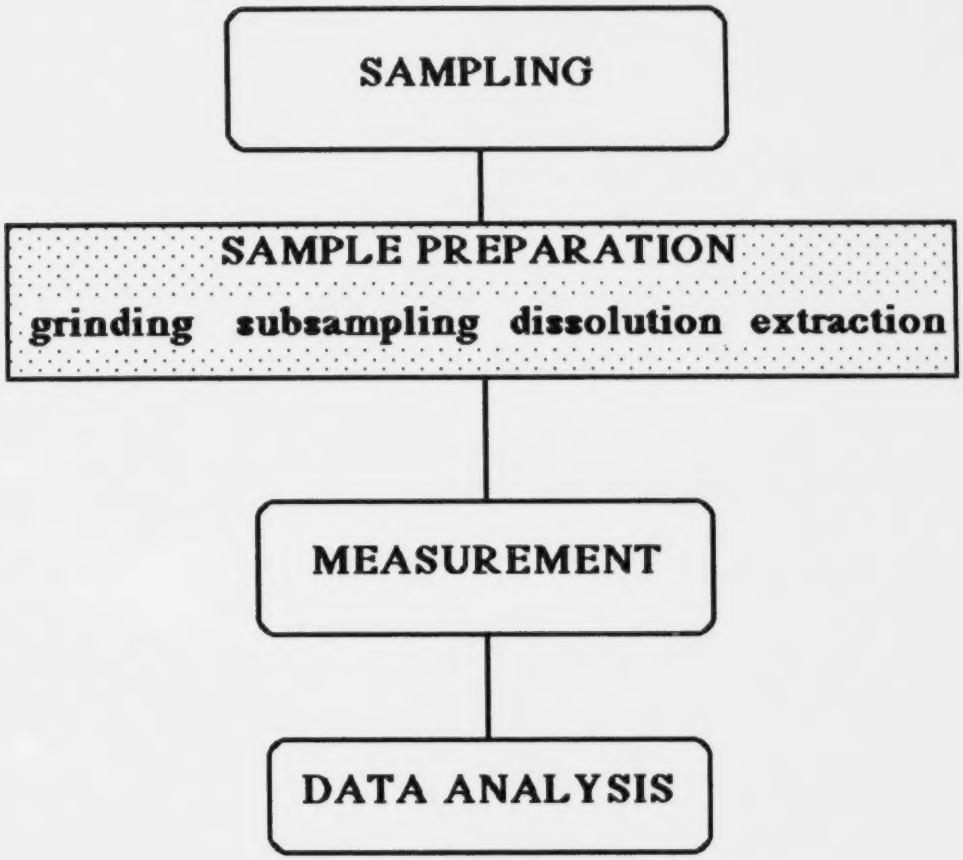


Figure 1.1 Overall Scheme for Elemental Analysis.

Wet ashing procedures almost always incorporate oxidizing reagents to complete the digestion.[49] Most chemical digestion procedures for biological materials rely on the use of H_2O_2, HNO_3 and/or $HClO_4$ to decompose the organic matter. HF is used to decompose siliceous matter that might be present in the sample, through formation of volatile SiF_4, whereas HCl and H_2SO_4 merely serve as strong acids.[50]

Traditionally, heat is used to speed up the process of wet acid digestion. In conventional "hot plate" digestions, the sample plus acid is placed in an open beaker on top of a hot plate in a fume hood. To achieve complete dissolution of biological materials, acids and hydrogen peroxide can be used either singly or in combination, depending on the nature of the sample and the analyte of interest. For example, during acid decomposition of human blood and plasma for the determination of selenium, the use of HCl is discouraged because of the loss during digestion of volatile Se_2Cl_2, $SeCl_4$, $SeOCl_2$ and SeO_2.2HCl.[51] Similarly, the volatility of arsine is the reason why the use of HCl is discouraged when plant material is dissolved for the measurement of arsenic.[49]

Nitric acid is used widely in organic decompositions because of its highly oxidizing nature and solubility of nitrates. However, it has a low boiling point, so that temperatures of 250 to 300 °C needed for some applications cannot be achieved in open vessel digestions unless sulfuric acid is added to elevate the boiling point. Because of its superior oxidizing power when hot and concentrated, perchloric acid is often selected for "hot-plate" wet digestion of plant and animal tissues. Evans *et al.*[52] described a method for the acid digestion of Cd, Ni and Pb from foodstuffs using nitric and sulfuric acids in Kjeldahl flasks. For high-fat samples, the addition of perchloric acid to complete the digestion was necessary. The use of perchloric acid, however, carries with it safety concerns because of its hazardous nature.[53,54] It is therefore essential that

special safety precautions are taken in its use. For example, close supervision during digestion is required, and the use of special fume hoods equipped with wash down facilities is essential.[55]

In a majority of cases, complete dissolution using the traditional approach takes a very long time to accomplish. For example, a nitric/perchloric acid procedure for complete dissolution of liver samples developed by Johnson *et al.*[56] takes between 15 and 23 hours, and a hydrogen peroxide/nitric/perchloric acid procedure developed by Hall and Gupta[57] for complete mineralization of plant material takes between 22 and 30 hrs.

1.2.3 Modern Methods of Dissolution

Dissolution using traditional hot-plate procedures is now recognized as one area in the analytical laboratory that limits productivity.[58,59] Figure 1.2 shows the relative distribution of analysis time among sample processing (primarily dissolution), instrumental measurement, and data processing. It can be seen that sample preparation alone takes about 66% of the total analysis time.

In addition, dissolution is critical from the data processing point of view. The technique used for this purpose must ensure that all of the analyte goes into the solution phase, and that contamination by reagents does not occur.[60] If this is not achieved, errors in the analysis will arise. Figure 1.3 shows the error of each step relative to the total error. Sample preparation using traditional methods clearly is the major contributor of error in the analysis.[58]

1.2.3.1 Hot-Block Digestors

Driven by the need to overcome the limitations of conventional digestion methods, several alternatives have been investigated. One of these is chemical digestion in a thermally heated metal block.

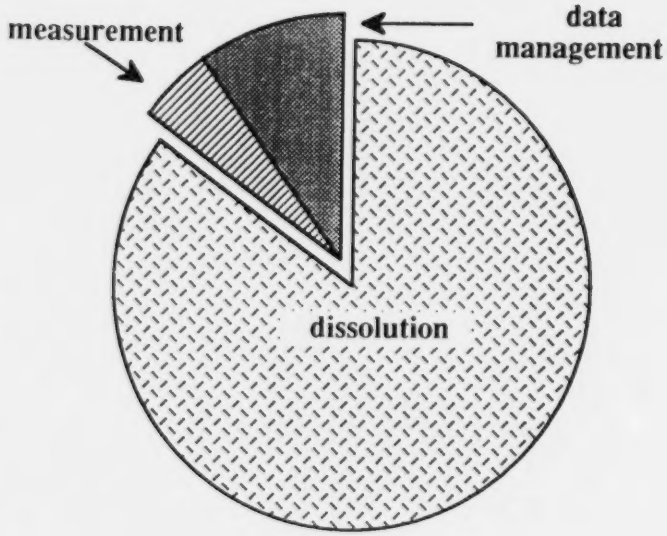


Figure 1.2 Time Taken to Complete Sample Dissolution in Relation to Total Analysis Time[58]

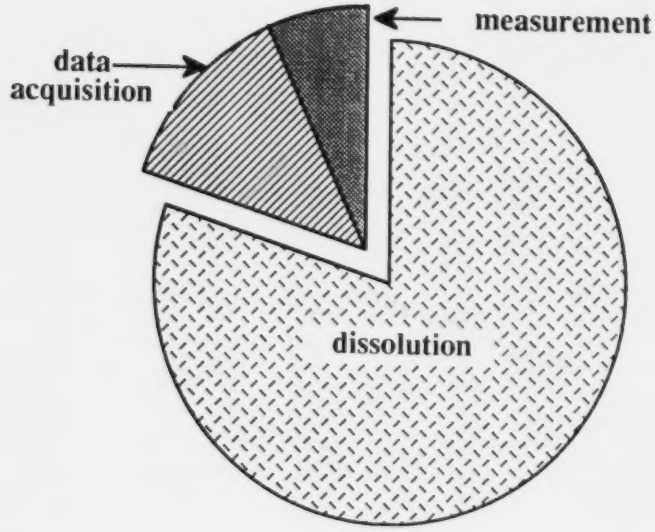


Figure 1.3 Source of Errors in Sample Analysis[58]

The introduction of hot-block digestors in the early 70's[61,62] enabled laboratories to process a large number of samples within a short period of time. Agemain *et al.*[63] developed a hot-block digestion procedure in which samples were placed in 18 calibrated test-tubes. These tubes were fitted into holes drilled in an aluminum block that was held at 150 °C. Dissolution in peroxide/nitric/sulfuric mixtures was found to be rapid, reducing total digestion time to 3 hours. This was attributed to uniform heating of the sample compared to conventional hot-plate procedures. The digestion rate may be controlled through a combination of well depth, residence time and block temperature.[62,64] More recently, microprocessor-controlled block digestors have been developed and are available commercially. A commercial unit utilizing robotic automation for digestion of water samples has been described.[65] The unit was evaluated[66] and found to yield very good precision in the determination of a variety of metals. With these digestors, up to 50 samples can be simultaneously digested in less than an hour. Time and money are both saved, while reproducibility and safety are enhanced.

In the 1980's, block-digestion was rapidly replaced by closed vessel procedures. The reason for the change was that, like conventional hot-plate procedures, block digestion is basically an open vessel technique, so that loss of analyte during digestion is still a major concern.

1.2.3.2 Closed-Vessel Acid Digestion

Closed vessel decomposition techniques were introduced to prevent the loss of analytes encountered during open vessel digestions. In addition, closed vessel procedures, typically using teflon-lined steel bombs with screw caps,[67,68] can operate under high pressure, enabling digestion mixtures to work at much higher temperatures than is possible with open vessels. Digestions at temperatures of

100 degrees above those used in traditional procedures are not uncommon.[69,70] This significantly reduces the time required to complete the digestion. Recently a high pressure-high temperature digestion system was described[71] in which operating conditions as high as 320 °C and 2000 psi are utilized in the digestion of many types of difficult samples.

Matusiewicz[72]and Jackwerth *et al.*[73] used Teflon bombs to decompose organic and inorganic materials rapidly. Matusiewicz *et al.*[74] later described a bomb digestion procedure that uses only hydrogen peroxide to complete dissolution of plant material in less than 4 hours.

Katz *et al.*[75] compared the performance of dry ashing, conventional open vessel, and closed vessel bomb techniques for the dissolution of sewage sludges. Dry ashing was found to be the least efficient in terms of element recovery. Digestion bombs were found to give higher values for Cd, Pb, Cu, Fe and Ni when compared to open vessel techniques, and to make digestion occur more rapidly. Bernas[76] and Langmyhr[77] described the advantages of closed systems, and concluded that these were superior to conventional open-vessel techniques.

1.2.3.3 Closed Vessel Microwave Digestion

It was not until 1975 that a microwave energy source for analytical sample dissolution was introduced. In that year, Abu-Sarma *et al.*[78] studied the use of microwave energy to speed up the dissolution process. The procedure is similar to the one described in Section 1.2.3.2 because the same type of closed vessel is used. The only difference is that instead of using thermal ovens to provide heat, these procedures use microwave ovens as the source of energy. Commercially available ovens operating at 2450 MHz and several hundred watts of electrical energy are commonly used, and microwave-transparent teflon bombs are employed as vessels for dissolution.[79]

The application of microwaves to sample/acid mixtures produces about 5 billion molecular collisions per second, and elevated pressures inside the vessels cause rapid dissolution because the acid mixtures can act at much higher temperatures than the normal boiling points at atmospheric pressure. In conventional heating, only the surface is directly heated, and the heat must be conducted from the surface to the interior. In microwave heating, the uptake of microwave energy is essentially instantaneous throughout the entire sample, and the heat conduction stage is eliminated.[80] The result is reduction in sample digestion times from hours to minutes.[81] A similar comparison with aluminum block digestors shows microwave techniques to be faster, and to provide more controllable and reproducible conditions.[82] The technique has been successfully applied to a variety of matrices, including soils,[55,83] sediments,[84] sewage sludges,[85] biological materials,[86] brewing materials,[87] and agricultural crops[79,88], as well as diet and fecal samples.[89] Zehr *et al.*[90] recently introduced the use of basic solvents in place of acids for microwave digestion. This further extends the range of applicability of the method to materials containing CoO, MoO_2, ThO_2 and W_2O_5, compounds which are insoluble in a typical acid microwave digestion.

There is little doubt that microwave acid digestion techniques have revolutionized sample dissolution. With microwave heating, sample preparation is safer, less costly, and needs minimal supervision.[91-93] In addition to the advantage of speed, microwave techniques can be fully automated. Grillo *et al.*[94] have recently described an integrated microwave digestion system, complete with a notebook computer and a printer, for dissolution of samples prior to AA, ICP-OES, and ICP-MS. The method has been adopted by the EPA for certain applications, including aqueous samples,[95] sediments, sludges, soils and oils.[96]

There are several shortcomings in the microwave dissolution technique. First, microwave heating is material-specific in that only those molecules that absorb microwave energy can generate heat.[97] Therefore, when microwaves pass through materials that are either transparent to or reflect microwaves at 2450 MHz, no heat is generated.[98-101] Because of this material specificity, certain matrices (plastic, paper, silica, carbonate, sulfur, etc.) may not be affected. Secondly, as in closed vessel bomb techniques, safety of the method is undermined by pressure build-up inside the vessel during the digestion process.[102] The ratio of sample to reagent must be strictly controlled, and occasional venting of the digestion vessel needs to be done unless in-built pressure release mechanisms are incorporated.[103] As a result, automation of microwave digestion procedures suffers from many technical problems.[104]

The Fall 1990 issue of Environmental Lab Automation Notes[105] describes an automated microwave digestion system that uses a robotic arm to dispense acid into a digestion vessel, cap the vessel and place it in a microwave oven. The robotic arm also removes the vessel from the oven and vents it as defined by the protocol for digestion. Because the system is completely automated, precision and accuracy are improved as compared to hot-plate procedures because exact timing of the steps is maintained, safety is improved because personnel are not exposed to corrosive acids, and labor requirements are reduced, freeing staff for non-routine tasks. The problem with this approach is the cost of computers and robotic arms. As a result, several alternatives are currently being investigated, including stopped-flow methods.[106]

Despite these shortcomings, the introduction of microwave techniques represent the only major advance in dissolution methods over the past four decades or so.

1.3 Alternative Approaches to The Problem of Sample Dissolution for Elemental Analysis

1.3.1 Background

Although modern analysis techniques are all capable of providing highly reproducible determinations on a routine basis, it can be seen from the previous sections that methods of sample preparation do not always match the performance, throughput, or economy of the analyses.[71] Indeed, analytical chemists have spent considerable effort on the development of methods having improved sensitivity, accuracy and precision, but not as much time on the steps preceding the measurement operations. Dissolution using conventional hot-plate methods is often costly, hazardous, slow and inconvenient. Because of this, the overall analysis time is increased even if super computers are used to run modern analytical instruments. While the microwave technique gives remarkable improvement for many materials, there are many other situations in which the matrix is not appreciably affected by microwave heating. Consequently, dissolution is now widely regarded as a problem for which modern analytical chemists must find immediate solutions.

1.3.2 Avoiding Dissolution by the Direct Analysis of Solids

There are two options open to the analytical chemist in tackling the "dissolution problem"; either eliminate dissolution altogether, or find better alternatives to existing technologies. To eliminate dissolution from the overall analytical procedure, techniques for introducing the solid sample directly into instruments such as the ICP or AAS must be sought. The two most widely investigated techniques for direct analysis of solid samples are slurry atomization and direct sample insertion. A review of current trends in these areas follows.

1.3.2.1 Slurry Techniques

There are numerous reports in the literature dealing with direct analysis of solid samples by electrothermal vaporization.[107] In this technique, an aqueous suspension of the solid is first pipetted onto a graphite platform and placed in a graphite tube furnace. Electrothermal vaporization is then performed on the solid sample to atomize the desired elements. Miller-Ihli[108] describes a method for the determination of several elements in slurries of biological reference samples using this approach. Good agreement between experimental and certified values for several elements was obtained. Jackson *et al.*[109] pipetted 20 µL aliquots of soil/water slurry onto a graphite furnace platform for the determination of Pb by AAS. Reasonable accuracy was reported. Dudas *et al.*[110] aspirated clay suspensions into an ICP-OES instrument without sample dissolution. Good agreement was obtained between electron microprobe results and the ICP-OES method. Williams *et al.*[111] used the same approach for analysis by ICP-MS of finely ground soils and industrial oxide catalysts. More recently, Cabrera *et al.*[112] used slurry GFAAS to determine Cd in various types of sea food. Reliability and accuracy were good.

1.3.2.2 Solid Sample Insertion Methods

In atomic spectroscopic methods, the most common way of introducing dissolved samples to a flame (FAAS) or plasma (ICP) is by pneumatic nebulization. Browner and Boorn[113] note that after spending up to several hours dissolving a sample, 99% of it never reaches the atomizer because of the low efficiency of this sample introduction method. They also note that by introducing the sample directly to the atomizer, tedious and time consuming dissolution procedures are avoided, and wasteful nebulization is eliminated altogether.

Techniques for direct sample insertion date back to the beginnings of practical spectrochemical analysis when Kirchhoff and Bunsen[114] proposed the wire-in-flame technique. In 1979, Horlick and Salin[115] described a "Direct Sample Insertion device" that made direct introduction of discrete samples into an ICP possible. Linear calibration curves were obtained for zinc powders. Clearly, direct analysis of solids eliminates the problems encountered during dissolution. It reduces sample preparation time, decreases the possibility of loss of analyte, and reduces the likelihood of contamination.

1.3.2.3 Current Problems with Direct Analysis of Solids

Techniques involving solid sampling introduce unique problems, however, that are not normally encountered when handling liquid samples. An obvious limitation is the requirement that a homogeneous distribution of particles be achieved prior to analysis. If this criterion is not met, problems with precision and accuracy are bound to arise. With slurry nebulization, precision may be very poor because of sampling errors and lack of reproducibility during the aliquoting step. In addition, slurry nebulization may lead to clogging of the nebulizer by solid particles. As much as a 25% reduction in aspiration rates can be encountered, requiring the use of time consuming standard additions procedures to correct for this variable.[110]

For direct sample insertion, small samples are used and one should be aware of possible sampling errors. Reproducibility is affected by the need for weighing sub-milligram quantities of solid material onto graphite platforms. The procedure is difficult, and requires considerable skill and effort, as well as sensitive balances. Particle size should be kept as small as possible to increase precision. For this purpose, additional grinding procedures in special containers designed to minimize contamination may be necessary. This process is often tedious and time

consuming. In addition, preferential partitioning of analytes in the larger or smaller particles should be considered. Analyte may be lost during sieving or handling if the analyte prefers to partition in one size of particles. This is described in detail by Holcombe and Majidi.[116,117] Since it is difficult to separate sample vaporization and excitation processes, matrix effects are often observed, leading to non-linear curves, especially with non-volatile elements.[118] In addition, explosive vaporization and visible particles flying through the plasma have been observed in some cases,[119] which contribute to decreased accuracy and precision.

1.3.3 Summary of Trends is Sample Preparation Research

The examples cited in this introduction reveal a current trend towards development of methods that reduce sample preparation times to match the rapid measurement capabilities of modern analytical instruments. In order to achieve this, two approaches have been proposed. The first involves introducing alternate forms of energy to speed up the process, *i.e.* microwave techniques. The second involves eliminating dissolution altogether, *i.e.* direct solid analysis. Like most techniques at the infancy, direct solid analysis systems suffer from many technical problems. There is also lack of sufficient knowledge about the fundamental processes that occur when a solid is atomized in the flame or plasma. Until these problems are solved, liquid nebulization will remain an integral part of flame and ICP atomic spectrometry. And until such time as nebulization becomes obsolete, the demand for improved methods of analytical sample dissolution to replace or complement traditional methods will continue.

1.3.4 Background for Consideration of an Ultrasound-Based Dissolution System

It is for these reasons that an investigation was undertaken to study the behavior of chemical systems when exposed to ultrasound. The goal was to seek a process using this technique that would speed up sample dissolution for elemental analysis.

The next section provides background information and a survey of relevant literature on the use of ultrasound to promote chemical reactivity.

1.4 Ultrasound: A General Overview

Ultrasound is the name given to sound waves having frequencies higher than those to which the human ear can respond. Commonly encountered ranges of sound frequencies can be functionally divided into low frequency sound, power ultrasound and diagnostic ultrasound,[120] shown in Table 1.1.

Table 1.1 Classification of Sound Waves.

type	uses	frequency range
low frequency sound	human hearing	16 Hz - 16 kHz
power ultrasound	welding, cleaning	16 kHz - 100 kHz
diagnostic ultrasound	SONAR, sonography	100 kHz - 10 MHz

1.4.1 Properties of Ultrasound Waves

Ultrasound behaves like electromagnetic radiation in all respects except that, being a sound wave, it can only be propagated through a medium that possesses elastic properties. Propagation is in the form of successive rarefaction and compression phases.

These pressure waves may be described[121] by the equation:

$$P_t = P_A \sin 2\pi ft \quad \text{.....(1)}$$

where P_t is the pressure at time t, f is the frequency, and P_A is the amplitude of vibration. These pressure waves are sinusoidal in character, and, like electromagnetic waves, their speed c is related to wavelength λ by the expression:

$$c = f\lambda \quad \text{.....(2)}$$

Ultrasound intensity is related to the pressure by the expression:

$$I = P_A^2/(2\rho c) \text{.....(3)}$$

where ρ is the density of the medium.

Because of imperfect coupling of ultrasound waves between the source and most media through which they are propagated, ultrasound wave intensity I decreases with distance from the source x according to:

$$I = I_o \exp[-2\alpha x] \quad \text{.....(4)}$$

where I_o is the intensity at the source, and α is the coefficient of absorption of the waves in the medium.

The absorption coefficient is related to the viscosity η of the propagating medium by the relation:

$$\alpha = 2\rho^2 f^2/\eta\, c^3\{ (4\rho/3)+(\gamma-1)\kappa/\gamma C_v\} \quad .....(5)$$

where κ is the thermal conductivity, and γ is the polytropic ratio, *i.e.* the ratio of the heat capacity at constant pressure C_p to that at constant volume C_v.

1.4.2 Generation of Ultrasonic Waves

Ultrasound can be produced in a variety of ways. Galton[122] was the first to report on a method of producing ultrasound by means of a whistle. This method produces low-intensity ultrasound that does not affect chemical reactivity. The basis of present-day generation of ultrasound with sufficient intensity to enhance chemical reactivity is the piezoelectric (pressure-electricity or Curie) effect.[123,124] When a mechanical force is applied between two faces of a piezoelectric crystal, *e.g.* quartz, rochelle salt, ammonium dihydrogen phosphate or potasium dihydrogen phosphate, a potential is developed perpendicular to the applied force. The magnitude of this potential depends on the applied pressure. The polarity is reversed if a negative pressure is applied. To produce ultrasound, the reverse effect is used, *i.e.*, application of an alternating electromotive force at the crystal ends so that the crystal expands and contracts with the same frequency as the voltage supply. The most commonly used frequencies for power ultrasound are in the 20 to 100 kHz range. In order to generate ultrasound at 20 kHz, a potential alternating at 20 kHz is applied to the crystal.

Modern ultrasound units use ferroelectric compounds, such as barium titanate $BaTiO_3$, lead metaniobate $PbNb_2O_6$, or most commonly a lead zirconate titanate $PbZrTiO_3$ (PZT) mixed crystal in ceramic form, to produce ultrasound. These ceramic materials produce ultrasound most efficiently at their natural resonance frequency. This optimum frequency depends not only on the resonating material, but also on its geometric properties such as size and orientation. Because it is not practical to alter these properties once a transducer has been fabricated, most commercial units operate at a fixed frequency.

1.4.3 Ultrasound Applications in Industry, Biology and Medicine

Ranging by SONAR[125] and medical imaging by sonography[126] are probably the most common applications of ultrasound based on pulse-echo principles. This enables cardiologists to detect thickened or obstructed arteries, and also to identify lesions and cysts. Obstetricians can monitor the development of a fetus, and may even guide surgical tools though delicate fetal organs. Diagnostic ultrasound uses high frequency-low power energy for enhanced resolution.

For non-diagnostic applications lower frequencies are used, but the ultrasound is generally of high intensity.[127,128] Blood clots can be dissolved, kidney stones pulverised, and surgical instruments and teeth cleaned efficiently.

In biology and biochemistry, high intensity ultrasound is used to disrupt cell walls. Webster and Stone[129] recently described a procedure for isolating cell walls after disruption of root hairs using ultrasound. With ultrasound, spores may be efficiently removed from their sacs, and cellular organelles, enzymes, protein molecules and nucleic acids can be released more quickly from cells without damage or denaturation.[130,131] Sonication for longer times can cause fragmentation of certain biological compounds, as well as solubilization of others.[132,133]

In addition to applications in non-destructive testing, drilling, grinding, cutting and welding, high power ultrasound will eventually find its way into the home. A liquid bombarded with ultrasonic energy breaks up into microscopic bubbles that aid in mechanical removal of dirt from crevices. This approach is used to manufacture ultrasound-based washing machines. A spokesman for Branson Ultrasonics Corp. recently predicted that a dishwasher load may take only a couple of minutes and use a fraction of the water consumed by conventional washing machines.[134]

1.4.4 Ultrasound Applications in Chemistry

Perhaps the most common uses of ultrasound in the chemistry laboratory are cleaning of apparatus and dispersion of solids in solution.[110] In addition, filtration and extraction processes can be enhanced, smaller and more uniform crystals can be produced, and HPLC solvents can be degassed more efficiently in the presence of ultrasound.[135-139]

More recently, sonochemistry (using sound energy to carry out chemical reactions) has been introduced.[140] The origins of this technique date back to the late 1920's when Richard and Loomis[141] observed that if ultrasound is passed through iodide solutions, iodine gas is formed. Recent ESR studies[142] show that the reaction is caused by the production of highly oxidizing hydroxyl radicals when ultrasound of sufficient intensity is propagated into aqueous systems.

1.4.4.1 Ultrasound Synthesis of Organic Compounds

Although the exciting potential of ultrasound in the synthesis of compounds was proposed by Renaud in 1950,[143] the chemical literature has very few reports on this topic between that time and 1975. Part of the reason is that no

significant technological breakthroughs were made in the technology of ultrasound production.[144]

It was not until around 1980 that interest in ultrasound-assisted reactions began to increase. In 1978, Fry *et al.*[145] reported on the ultrasound-assisted synthesis of cycloalkanones from α,α'-dibrocycloalkanones. Since then, work in the field of ultrasound-assisted synthesis has expanded, and organic synthesis is by far the dominant application of ultrasound in chemistry. Hydrolysis reactions[146,147] and Strecker synthesis of amino nitriles[148] are examples of homogeneous ultrasound reactions. In the presence of metal catalysts, sonication has been found to increase rates of some reactions several-fold. For example, Mason *et al.*[149]found that chemical reactivity during the Ullmann diaryl synthesis is much higher in the presence of ultrasound, and Luche and Damiano[150] reported that in the presence of ultrasound the Barbier synthesis of alcohols takes only a few minutes to complete, whereas conventional methods take several hours. Boudjouk and Han[151] were able to achieve coupling of chlorosilanes only when ultrasound was used. Ando *et al.*[152]reported that the yields of ketones produced by oxidation of secondary alcohols are much higher than those obtained under mechanical agitation.

1.4.4.2 Ultrasound Synthesis of Inorganic Compounds

The application of ultrasound in the synthesis of inorganic compounds has also received considerable attention. Suslick *et al.*[153] reported on a method of metal cluster synthesis with the aid of ultrasound. Rates were found to be 100,000 times higher than in the absence of ultrasound. Several reports on the synthesis of reactive transition metal powders with the aid of ultrasound have recently appeared.[154,155]

The application of ultrasound clearly provides a different path from traditional sources of energy for the enhancement of chemical reactions. Enhancement may be observed in yield, acceleration of rates, or in changing mechanistic pathways of reactions to produce different products.

1.4.4.3 Ultrasound Polymer Degradation

It is also interesting to note that polymer degradation was among the first applications of ultrasound in chemistry. As early as 1933 Szalay,[156] Gyorgi,[157] Flosdorf and Chambers[158] reported that the viscosity of solutions of agar, starch and gelatin was reduced upon application of ultrasound. That these changes are largely caused by breakage of bonds to produce lower molecular weight compounds has been confirmed by recent studies.[159,160]

The proposal that ultrasound can reduce the time required to achieve dissolution of biological samples is based on utilizing such processes to promote decomposition reactions, especially those involving chemically resistant polymers such as cellulose.[161]

1.4.4.4 Applications of Ultrasound in Analytical Chemistry

Ultrasonic baths have been part of the analytical chemistry laboratory for a long time. Their use, however, has until recently been restricted to the cleaning of soiled glassware. More recent uses in analytical applications include nebulization, mixing and extraction.

1.4.4.4.1 Ultrasonic Nebulization and Slurry Stabilization

The use of ultrasound for nebulization of solutions has received increasing attention in recent years.[162] Ultrasound nebulization offers long term stability,[163] uniform drop size[164] and elimination of nebulizer plugging.[165]

Hieftje *et al.*[166] developed an efficient and inexpensive (obtained from a common room humidifier) ultrasonic nebulizer for ICP and microwave plasma-OES. Aerosol generation rates were found to be higher than those obtained by pneumatic nebulizers.

Ultrasound has been shown to be more efficient than mechanical vortex mixing for the stabilization of slurries prior to measurement. Edwards *et al.*[167] reported on an ultrasound method to disperse soil samples in distilled water using a probe-type vibrator. Reproducibility was found to be superior to vortex mixing. Miller-Ihli[168,169] developed an automated ultrasonic mixing accessory for slurry sampling into a graphite furnace atomic absorption spectrometer. The reported precision for biological samples was between 8 and 24%. Epstein *et al.*[170] applied this technique to determine Pb and As in river sediments. Van den Akker *et al.*[171] later used the same approach in the determination of heavy metals in sediments. Good agreement with expected values was obtained.

1.4.4.4.2 Ultrasonic Extraction of Metals

The review of Suslick[172] describes the applications of ultrasound to chemical synthesis and for initiation and enhancement of catalytic reactions. Since most sonochemical applications to date are mainly in the field of synthesis, no mention is made in the review of dissolution processes that might be applicable to chemical analysis.

There have been a few preliminary explorations in this area. Kumina *et al.*[173] has reported that ultrasonic treatment of plant materials can shorten preparation times over dry ashing or acid digestion. Eight metals were subsequently determined by atomic absorption spectroscopy. Complete dissolution was not achieved with three 4-minute extractions with dilute HCl, but the metal concentrations were as high or higher than with other dissolution techniques.

Precision was only fair. Karyakin *et al.*[174] also applied ultrasound treatment to soils for extraction of trace metals with HCl and/or HNO_3. The results for eight elements agreed only approximately with those found by conventional methods, but the time of extraction was reduced from 16 hours to 30 minutes. Details of the experimental procedures were scant. Because total dissolution was not achieved, in some instances metals bound to refractive minerals were found to be 100 times lower than expected.

As early as 1980[175] it was speculated that ultrasonic dispersion of soils in water at room temperature causes significant dissolution of certain analytes into the liquid phase. In 1989, Escudey *et al.*[176] reported on apparent dissolution during ultrasonic dispersion of soils; Al, Si and Fe were determined by atomic absorption spectroscopy. Evaluation of accuracy was not possible because sonication results were not compared to a reference method. The authors concede that the reported results are probably in error, since it appears that the presence in the flame of fine suspended clay particles from ultrasound dispersion likely produced artificially high results. In 1989, Akcay and Savasci[177] described the effect of ultrasonication on extraction rates and recoveries of strontium from river sediment. Ultrasonic extraction results were comparable to those of conventional procedures for adsorbed Sr, but the time required for extraction was reduced from an hour to 10 minutes. The reported precision was about 25%. In addition, total Sr was found to be low when compared to certified values. The authors attribute this to the siliceous matrix, which would require HF to destroy. From these reports it is clear that for sediments, ultrasound will effectively remove adsorbed metals.

When Miller-Ihli introduced the ultrasound device for slurry sampling,[168] it was noted that ultrasonic agitation for brief periods in dilute HNO_3 caused extraction of many metals into the liquid phase of the slurry, and this contributed

to the improved accuracy and precision over vortex mixing or air bubbling. Even though this may be of benefit to analytical sample dissolution, no reports have been published that investigate the mechanisms by which the extraction occurs.

A recent study by Saleh and coworkers[178] provides valuable insight into possible mechanisms of extraction. Ultrasonic cavitation was found to be responsible for the rapid extraction of Cu and Fe from palm oil into nitric acid solutions. The method was found to be applicable to liquid oil samples, but failed when solid oil samples were insonated. In their work, no attempt was made to use ultrasound to achieve total dissolution.

1.5 Summary and Goals of this Study

1.5.1 Summary

From the above reports, it is clear that there is a growing demand for new methods of analytical sample dissolution. In the interest of speed, economy and simplicity, a method that would simultaneously extract many elements, as well as allow the digestion of a large number of samples in a short time, is desired. Although ultrasound is widely used in the analytical laboratory for nebulization, as well as an aid to slurry homogenization in GFAAS, the idea that analytical sample decomposition might be performed using ultrasound has not been investigated.

1.5.2 Aims and Objectives

The purpose of this work is to study the conditions necessary for this technique to be applied to the elemental analysis of biological materials. To do this, the physical and chemical conditions under which rapid and quantitative dissolution occurs must be investigated. Principles of cavitation, sound

propagation, attenuation, and ultrasonic engineering need to be applied to develop the method.

In order to put ultrasound in perspective, the theory of cavitation is first reviewed in Chapter II, with special emphasis on the mechanical effects of ultrasound, especially in two-phase liquid-solid systems. This is then followed, in Chapter III, by an investigation of the chemical effects of ultrasound when passed into water. In this part of the study, the effects of gases during sonication, of the solvent, of solute concentration, and of ultrasound power, are investigated.

A protocol for ultrasound dissolution, designed on the basis of the preliminary studies outlined in Chapters II and III, will be developed in Chapter IV. The effect of solvent composition on aqueous biological solid suspensions will be investigated, especially in the decomposition of organic matricies to release elements that can be directly measured by atomic spectrometric methods.

Results of a study on the range of applicability of the method developed in Chapter IV will be presented in Chapter V. Examples include environmental, agricultural, food and clinical samples. Results for elemental analyses of certified reference materials from Canada, USA and Japan will be given to show the reliability and validity of the proposed technique.

Chapter VI will present results of a study of contamination introduced by the sonication probe, followed by an evaluation of a sonic horn designed for analytical sample dissolution.

CHAPTER II

STUDY OF PROCESSES THAT OCCUR ON APPLICATION OF ULTRASOUND ENERGY TO TWO-PHASE LIQUID-SOLID SYSTEMS

2.1 INTRODUCTION

In Chapter I, the basic concepts of ultrasound energy were reviewed. In this chapter, the physical effects of ultrasound when propagated into two-phase liquid-solid systems will be investigated. Since most of the physical phenomena observed when ultrasound passes through a liquid medium arise from cavitation, it is perhaps proper to review the theory of cavitation before the results obtained in this study are discussed.

2.1.1 Acoustic Cavitation

The idea of cavitation was conceived by Lord Rayleigh,[179] who defined it as a process whereby bubbles or voids are first formed in a liquid medium by some mechanical force acting to separate the liquid molecules, and then are forced to collapse.

Acoustic cavitation is a form of cavitation induced by ultrasonic waves. During rarefaction (the negative pressure cycle of ultrasonic waves), cavitation bubbles are formed and forced to grow in size. These bubbles normally form in aqueous solution from dissolved atmospheric gases. The bubbles are then forced to collapse during the subsequent compression (positive pressure) cycle of the waves.[180]

Cavitation bubbles are therefore excited to a pulsation with the frequency of the sound field (stable cavities) until rectified diffusion[181] ultimately causes large bubbles to fragment into smaller ones (transient cavities).

At sufficiently high ultrasound intensities (equation 3), the collapse of cavitating bubbles can be very violent, leading to extreme conditions of localized pressure and temperature. Theoretical calculations[182] using equations 6 and 7 predict temperatures of several thousand degrees and pressures of several thousand atmospheres to arise from a single collapse.

$$P_{max} = P[\frac{P_m(\gamma-1)}{P}]^{\gamma/\gamma-1} \quad \text{.....(6)}$$

$$T_{max} = T_o P_m \frac{(\gamma-1)}{P} \quad \text{.....(7)}$$

In these equations, P_{max} and T_{max} are the maximum pressure and temperature during collapse, respectively, T_o the ambient temperature, P_m the liquid pressure, P the pressure inside the bubble prior to collapse, and γ the ratio of the specific heats ($\gamma = \frac{C_p}{C_v}$) of the gas inside the bubble.

These equations assume that at 20,000 Hz (oscillations of the ultrasound waves per second), cavitation collapse is extremely rapid, in fact so rapid that very little heat exchange takes place between a collapsing bubble and the surrounding liquid (i.e. an adiabatic compression). This causes the gas inside the bubble to be strongly heated as it is compressed.[183] Most of the observed unique physical and chemical phenomena in ultrasound irradiated liquids is attributed to these extreme conditions.

The magnitude of the violence of the collapse during compression is related to the intensity of the sound waves. Cavitation effects are thus directly proportional to ultrasound power.

The following section describes the various ways of producing ultrasound of sufficient intensity that the resulting cavitational effects can be used with advantage for sample dissolution.

2.1.2 Sources of High Intensity Ultrasound in the Laboratory

Except for laboratory ultrasonic cleaning baths, devices for producing relatively high sonic intensities are hardly ever found in an analytical chemistry laboratory. This is partly due to the fact that to speed up chemical reactions, chemists often prescribe heat, uv light or electrical discharge. Ultrasound is often considered only for stirring, for cleaning soiled glassware, or for nebulizers in atomic spectroscopy. A brief review of ways for producing ultrasound in the laboratory is outlined below.

Early experiments on ultrasonic studies were performed using home-made reactors in which a resonating crystal was immersed in oil between two electrodes. Wood and Loomis describe one such reactor[184] in which a 50,000 V potential was applied to a quartz crystal 8 cm long and 1 cm thick to produce ultrasound frequencies of several hundred thousand Hz. By keeping the voltage across the crystal constant, they could maintain a constant power output of several watts.

In the 1960's, commercial ultrasound units based on this original model became common for cleaning purposes in the laboratory. Ultrasound cleaning baths consist of several oscillators mounted on the underside of a stainless steel tray. The ultrasonic energy is transmitted from the source to the chemical system under investigation through the tray into the solvent. Because the transfer of ultrasound is indirect, low power densities (typically 0.1 to 1 W/cm^2) are obtained, and while these are of sufficient intensity for most cleaning and some

synthetic applications, they are not useful for applications that require high power outputs.[185]

Ultrasonic equipment manufacturers have begun to produce devices that might be adapted for chemical applications. Recently, high-intensity probes that efficiently deliver ultrasound directly to the chemical system have been commercialized for biological cell disruption. These probes deliver sufficient power to promote decomposition reactions such as those discussed in Chapter IV.[186] The probe used in this work is described in section 2.2 below.

2.1.3 Aims and Objectives of this Study

Cavitation is, in the first instance, a physical process. The unique physical processes which occur when a liquid medium is irradiated with ultrasound will be the focus of the study reported in this chapter.

A solid sample brought to an analytical laboratory for quantitative analysis is often dissolved in a solvent prior to measurement. The initial step in this process is formation of a heterogeneous two-phase solid-liquid system. All reactions between the liquid and solid phase occur at the surface of the solid. A significant factor controlling the rate of dissolution is surface area. In this study, the physical behavior of several solid materials when exposed to 20-kHz high intensity ultrasonic waves is investigated, and the results are compared with those obtained when heat energy alone is used.

2.2 EXPERIMENTAL SECTION

2.2.1 Equipment

2.2.1.1 Ultrasound Unit

The ultrasound unit used in this work is manufactured by Sonics and Materials Inc. (Danbury, CT, USA) and marketed by Ace Glass Inc. (Vineland, NJ, USA). A schematic diagram is shown in Figure 2.1. It consists of a power module (model 9810, 600W double channel) to convert 60 Hz line voltage to 20 kHz energy. The module is equipped with a microprocessor-based timer that allows the user to program the unit in terms of duty cycle and duration. Six ranges enable the timer to be set from a tenth of a second to 999 hours. The power output can be varied in 60W increments from 0 to 600W rms. The output of the power module is transmitted to a lead zirconate titanate ceramic transducer that converts the 20 kHz electrical signal to 20 kHz acoustic energy. The resulting ultrasonic waves are focused into the liquid through a 0.75" diameter direct-immersion horn fabricated from a Ti alloy. The end of the horn has a replaceable 0.75" diameter Ti alloy tip.

Sonications in this chapter were carried out in 50-mL borosilicate glass beakers which were raised or lowered with a laboratory jack. Both horn and sonication vessels were clamped onto a stand inside a steel enclosure lined with 2-inch thick sound deadening styrofoam. The front of the cabinet was a door of thick transparent Plexiglas to allow monitoring of the experiment. As an additional safety precaution, a pair of ear protectors, similar to those used for noise protection at airport runways, was worn at all times during the operation of the unit.

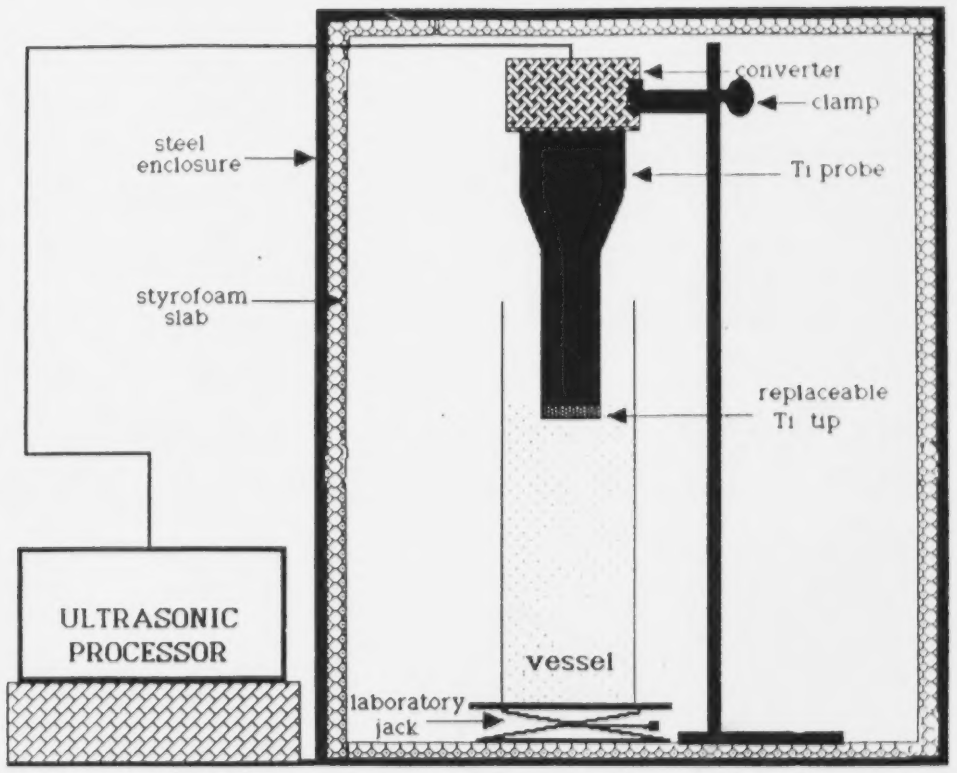


Figure 2.1 Ace Glass 9810 Assembly Used to Study Processes that Occur on Application of 20 kHz Ultrasound Energy to Two-Phase Solid-Liquid Systems.

2.2.1.2 Optical Microscopy

Sonicated solids were examined using an optical metallurgical microscope (Olympus BHT-M, Olympus Optical Co., Tokyo, Japan) in the laboratory of Prof. Jed Harrison (Department of Chemistry, University of Alberta). The microscope is equipped with a 6V-20W halogen lamp for reflected light observation, and a 35-mm OMPC Olympus photographic camera with in-built microprocessor for optimizing exposure time.

2.2.1.3 Scanning Electron Microscopy (SEM)

A Cambridge Stereoscan 350 scanning electron microscope with an energy dispersive X-ray analyzer in the University of Alberta Entomology Department was used for those experiments in which fine morphological details of the solids not easily discernible with light microscopy needed closer examination. All experiments were done with a primary beam voltage of 20 kV.

2.2.2 Reagents and Materials

The sonication medium was in-house distilled water that had been passed through a mixed strong acid/strong base ion exchange column (IWT, Rockford, Illinois, USA). HNO_3, NaOH, KI, and H_2NCONH_2 were reagent grade (BDH Chemicals, Toronto Canada); NH_4HF_2 (Fisher) and $Na_2S_2O_3$ (Anachemia Canada) were technical grade. Sonicated materials were calcium carbonate (classroom chalk), aluminum foil (Alcan, Canada), copper wire (99.9%), bovine hemin (Sigma Chemical Co., St. Louis, MO, USA), TLC-grade granular cellulose (Whatman Ltd., Kent, UK), and bovine liver SRM 1577a (National Institute of Standards and Technology, Gaithersburg, USA).

2.2.3 Methods

2.2.3.1 Sonication Procedure

Solids were transferred into sonication vessels. Approximately 40 mL of deionised water was added to each sample, then the horn was immersed in the water so that the tip was about 3 mm below the surface of the water and 4 cm above the bottom of the vessel. The power level and timer were than set at the desired levels and ultrasound applied in continuous (non-pulsed) mode.

2.2.3.2 Preparation of Samples for SEM Studies

Prior to SEM observation, sonicated solids were allowed to dry at room temperature for at least 2 days and mounted on the SEM sample platform. Unsonicated solids were mounted without further treatment. Non-conductive samples were plated with gold by sputtering under vacuum prior to observation.

2.2.3.3 Iodimetric Determination of Copper[187]

Solid residue from sonicated copper samples was dissolved in 10 mL of 6M HNO_3. The resulting solution was then quantitatively transferred to a 200-mL conical flask and boiled for 3 min to remove a majority of the nitrogen oxides evolved during dissolution in acid. Approximately 10 mL of deionized water and 5 mL of 4% urea solution was added and the solution boiled again for 1 min to remove any remaining nitrogen oxides. After cooling, 20 mL of water were added, followed by neutralization of residual nitric acid with approximately 15 mL of 2.5M NaOH. The pH of the solution was then brought to 4 by addition of 1 g of NH_4HF_2. Approximately 2 g of KI was added to liberate I_2, which was then titrated with 0.01M $Na_2S_2O_3$ solution (standardized with electrolytic Cu wire) until most of the brown coloration had disappeared. Freshly prepared

starch indicator was then added and the titration continued until the blue disappeared permanently.

2.3 RESULTS AND DISCUSSION

2.3.1 Evidence of Cavitation: Sonication of Chalk and Al Foil

Chalk and aluminum foil are probably the most commonly used indicators or dosimeters of cavitation.[188] Previous work on the sonication of these materials was done on ultrasound baths of low power, and most literature reports provide only semi-qualitative results. The work reported here details of the effects of the cavitational process on these substances when an ultrasound horn is used.

2.3.1.1 Sonication of Chalk

To study the effect of 20 kHz ultrasound on solids, several pieces of ordinary classroom chalk, 3 cm long and 1 cm in diameter, were placed at the bottom of a sonication vessel and 40 mL of distilled water added. The vessel was then placed inside the cabinet and the horn immersed in the water to a depth of 3 mm. The power level was set at "5%" and ultrasound applied in continuous (non-pulsed) mode. After sonication, the chalk was dried, then mounted on the Olympus light microscope for observation. Photomicrographs of the chalk, taken before and after sonication, are shown in Figure 2.2.

Prior to sonication, the surface is uniformly even (Figure 2.2(i)). As soon as sonication is initiated the chalk undergoes dramatic changes. There appear to be tiny explosions at the surface as material is rapidly removed. A photomicrograph of the surface after sonication for 1 sec, Figure 2.2(ii), shows that it is no longer even, but has been extensively pitted at the sites of the observed implosions.

Figure 2.2(i) Photomicrograph of Chalk Taken Before Irradiation with Ultrasound.

Figure 2.2(ii) Photomicrograph of Chalk Taken After Irradiation with Ultrasound for 1 sec.

Figure 2.2(iii) is a photomicrograph of the chalk after sonication for 5 sec. The pitting is more extensive than in Figure 2.2(ii). Sonication for 20 sec resulted in complete dispersion of the chalk into a cloudy suspension. When the power was increased to "100%", cavitation was so rapid that complete dispersion occurred within 5 sec. Figure 2.2(iv) shows the unchanged surface upon immersion of a similar piece of chalk in boiling water for 1 hr.

These observations show that the ultrasound energy is concentrated in localized sites on the chalk surface where cavitation occurred. The process was investigated further by replacing the chalk with Al foil.

2.3.1.2 Sonication of Al Foil

Circular pieces of aluminum (1.5" diameter) were cut from a roll of kitchen foil and placed in the bottom of the sonication vessel. Sonication was performed as described in section 2.3.1.1 at 100% power for 5 to 120 sec. After sonication the foil was allowed to dry at room temperature, then mounted on the optical microscope for examination.

Figure 2.3 shows examples of the physical transformations that occur upon insonation. The unsonicated surface is flat and featureless (Figure 2.3(i)); after 5 sec of sonication, the surface is pitted (Figure 2.3(ii)), indicating the occurrence of cavitation. The pits are circular, and grow outwards, away from the horn. They increased in both average size and number with sonication time.

The number of pits per square centimeter was estimated by counting under a microscope for sonication times from 5 to 120 sec. The results are shown in Table 2.1, along with average sizes of the pits. The size increases with sonication time. It is possible that several cavitation events (cycles of bubble growth and collapse) may occur at the same spot, and lead to fatigue and failure of the material.

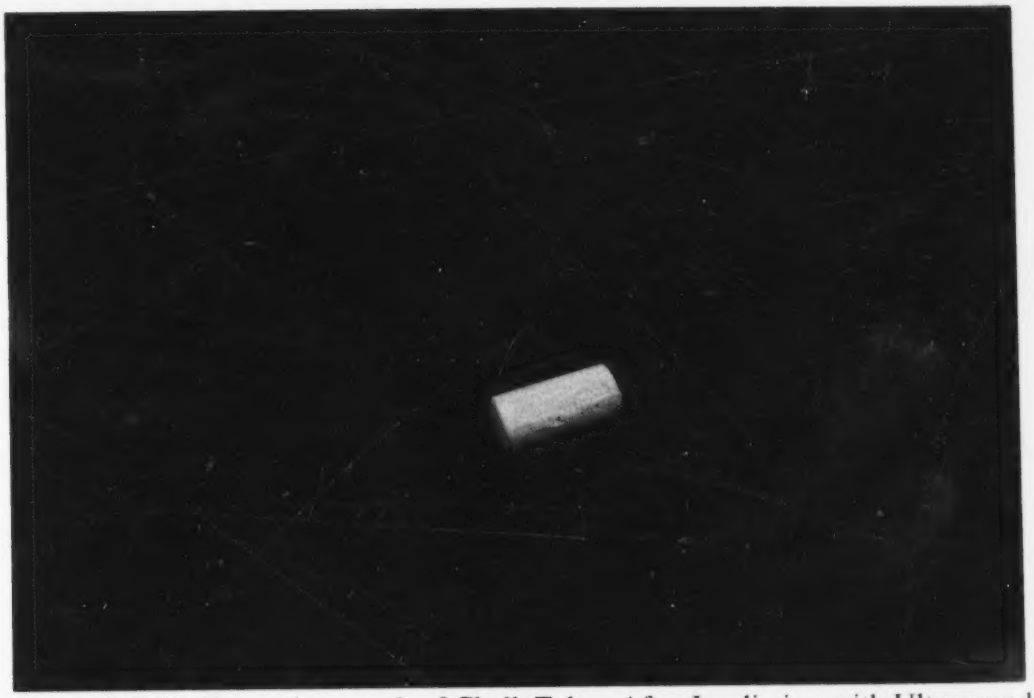


Figure 2.2(iii) Photomicrograph of Chalk Taken After Irradiation with Ultrasound for 5 sec.

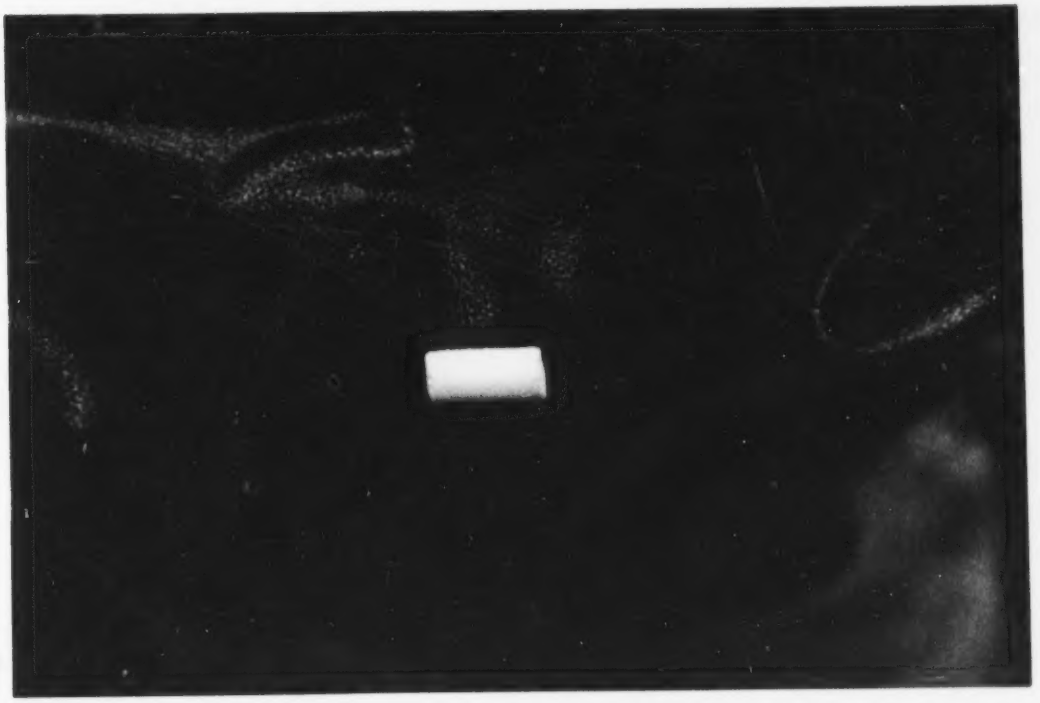


Figure 2.2(iv) Photomicrograph of Chalk Taken After Boiling in Water for 1 hr.

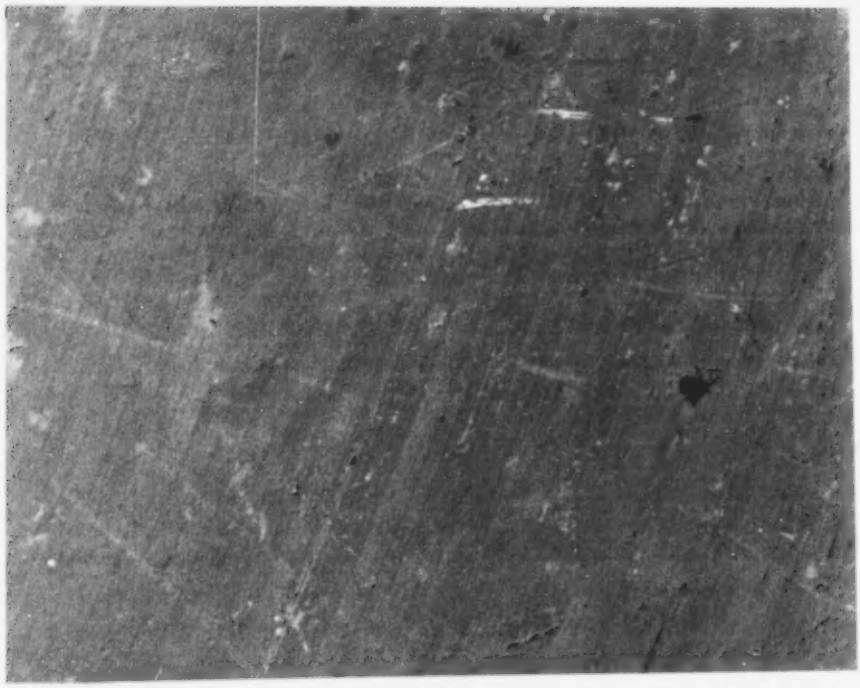


Figure 2.3 (i) Photomicrograph of Al Foil Taken Before Irradiation with Ultrasound.

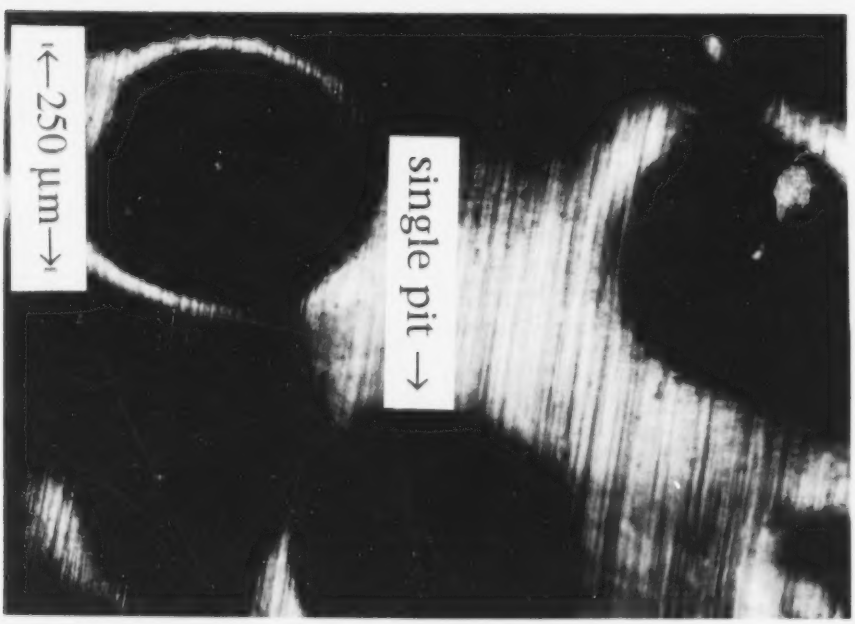


Figure 2.3 (ii) Photomicrograph of Al Foil Taken After Irradiation with Ultrasound for 5 sec.

Table 2.1 Effect of Sonication on Aluminum Foil.

sonication time (sec)	number of pits[1] per cm^2	pit dia.[2] (μm)
0	0 (uniform flat surface)	
5	190	240
20	510	320
60	550	440
120	extensive surface abrasion and erosion	

[1] Density of spots varies throughout the sonicated area. The reported values are averages of several counts; the associated standard deviation is about 20 pits per cm^2. Counting was done under 45X magnification, covering an area of 0.039 cm^2 per count.

[2] Values reported are averages. The diameters varied considerably; a typical standard deviation of 20 μm was observed.

Higher magnification, Figures 2.3(iii) and 2.3(iv), reveals the changes that occur to pits as sonication time is increased. The bottom of the pit appears to be the point where the surface of the metal first begins to show signs of cracking. There seems to be ejection of metal from the surface after 20 sec, and erosion is evident after 120 sec. Sonication beyond 120 sec results in fragmentation of the foil into small particles of Al, roughly 3 mm in diameter. These particles travel through the liquid phase at high velocities, driven by the turbulent action of the ultrasound energy.

2.3.2 Cavitation in Relation to Ultrasound Dissolution

The results presented here indicate that when ultrasound is propagated into an aqueous suspension of solids, the surface morphology of the suspended solid may be changed, with fragmentation and particle size reduction taking place. This means that the surface area of the solid is increased. Since the reaction of solvent with suspended solids during dissolution occurs at the surface, dissolutions in ultrasound have the potential of being faster than conventional thermal procedures because the solvent accessible surface area is enhanced. This is investigated in Chapter IV. The next section deals further with the physical effects of cavitational forces produced by an ultrasound horn.

2.3.3 Effect of Ultrasound Cavitation on Titanium and Copper Metal

Titanium and copper have high tensile strength. They were used in this study to evaluate the physical forces involved during cavitation. Titanium erosion was also of interest because the portion of the horn in contact with solvent is usually made from a titanium alloy.

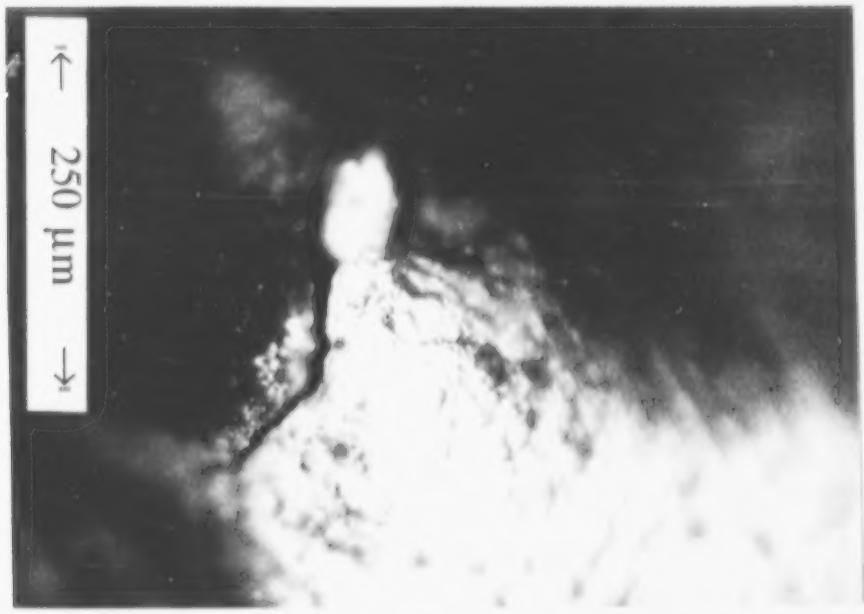


Figure 2.3(iii) Photomicrograph of Al Foil Taken After Irradiation with Ultrasound for 20 sec.

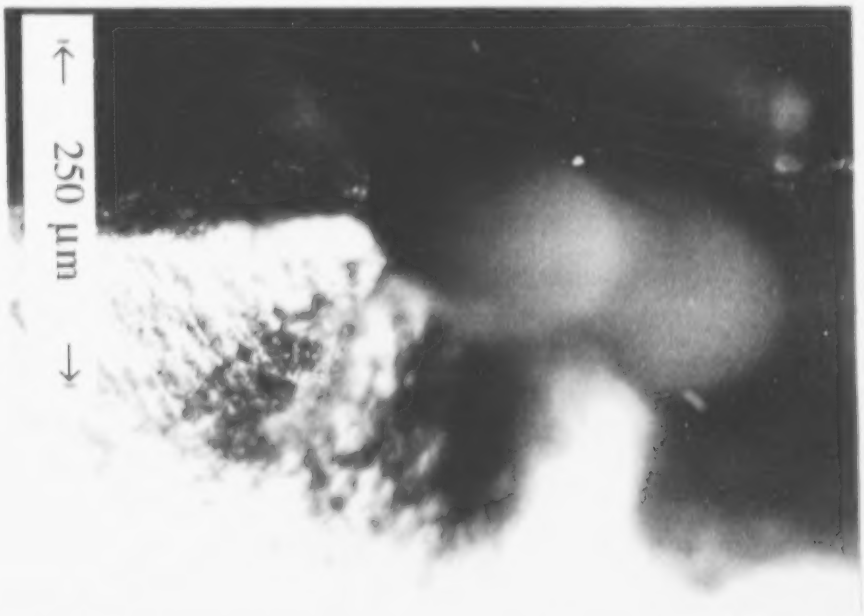


Figure 2.3(iv) Photomicrograph of Al Foil Taken After Irradiation with Ultrasound for 120 sec.

2.3.3.1 SEM Study of Erosion of a Commercial Titanium Alloy Horn Tip

According to equation 4, ultrasound intensity is greatest at the irradiating surface of the horn because the sound waves are unattenuated at this point. As a result, the surface of the tip can be expected to experience the harshest effects of cavitation. Two commercial titanium alloy tips were therefore selected as convenient samples for this study. The first was a new unused tip; the second had been used for 15 hours. These 15 hrs were logged during sonications of 40 mL of water at 100% power, for durations of not more than 3 hrs each time.

The tips were mounted, without treatment, on the SEM sample platform for microscopic observation. In Figure 2.4(i) the surface of a new Ti tip is shown. Except for machining marks, the surface is flat and featureless. On the other hand, the surface of a used tip shows numerous cracks and extensive erosion. This is shown in Figure 2.4(ii).

The forces involved during cavitation are energetic enough to rip metal fragments from the surface of the probe. An attempt was made to remove the cracks by polishing the eroded surface with fine emery cloth (3M, 413Q-400 size). The result is shown in Fig 2.4(iii). The erosion appears to have been very deep, and the characteristic circular patterns of cavitation are deeply etched on the surface.

2.3.3.2 Experiments with Copper Wire

To study the effect of 20kHz ultrasound on copper metal, several 0.9 g segments of pre-weighed metallic copper wire were placed in a sonication vessel, 40 mL of water added, and sonication performed at full power for 1 hr. The wires were then dried and weighed again. An average weight loss of 20 mg after sonication was recorded. The water in which the sonication was done was evaporated to dryness. A residue of metallic copper particles remained.

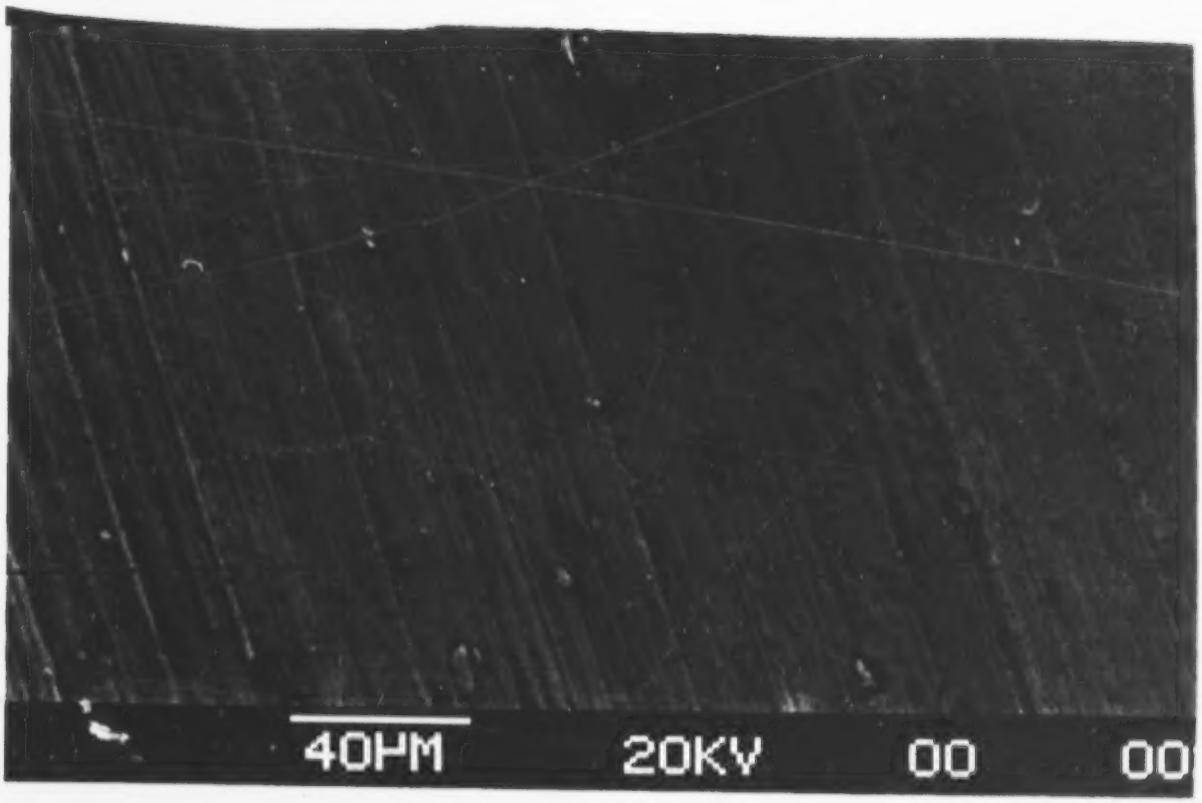


Figure 2.4 (i) SEM Photomicrograph of a New Ti Alloy Horn Tip.

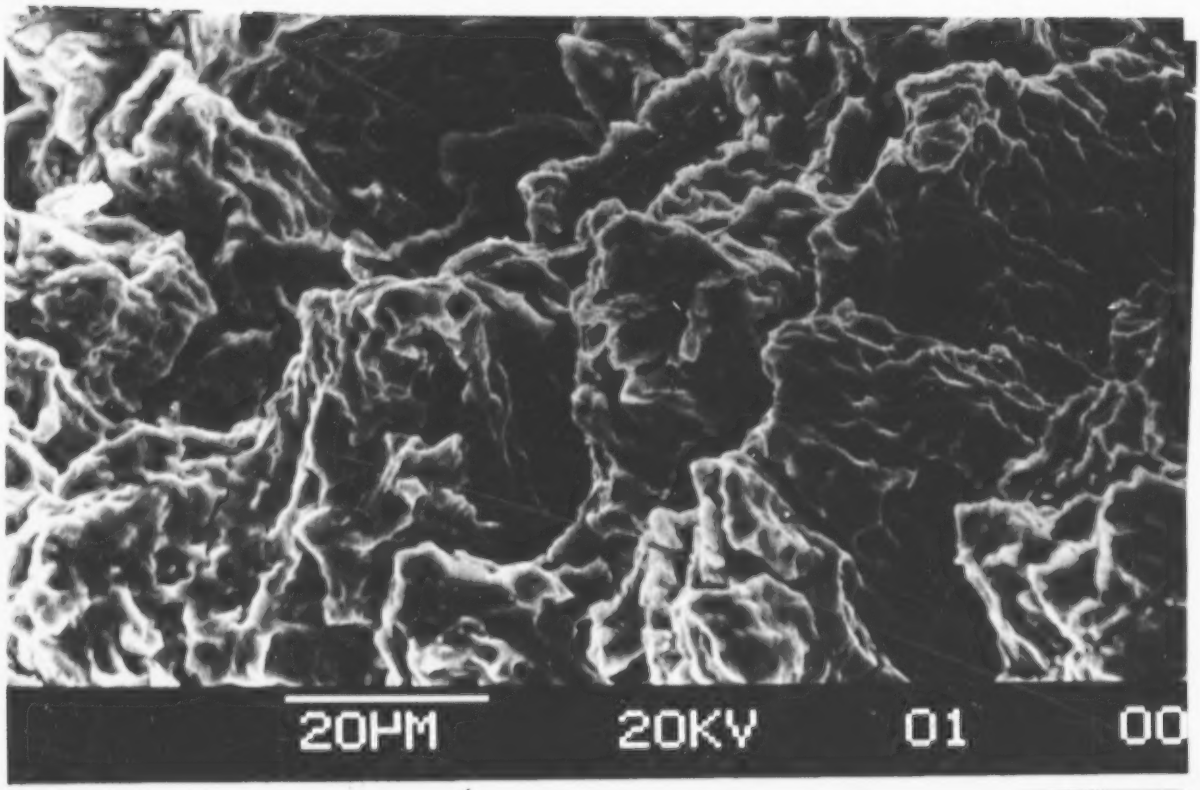


Figure 2.4 (ii) SEM Photomicrograph of a Ti Alloy Horn Tip After 15 hrs of Use.

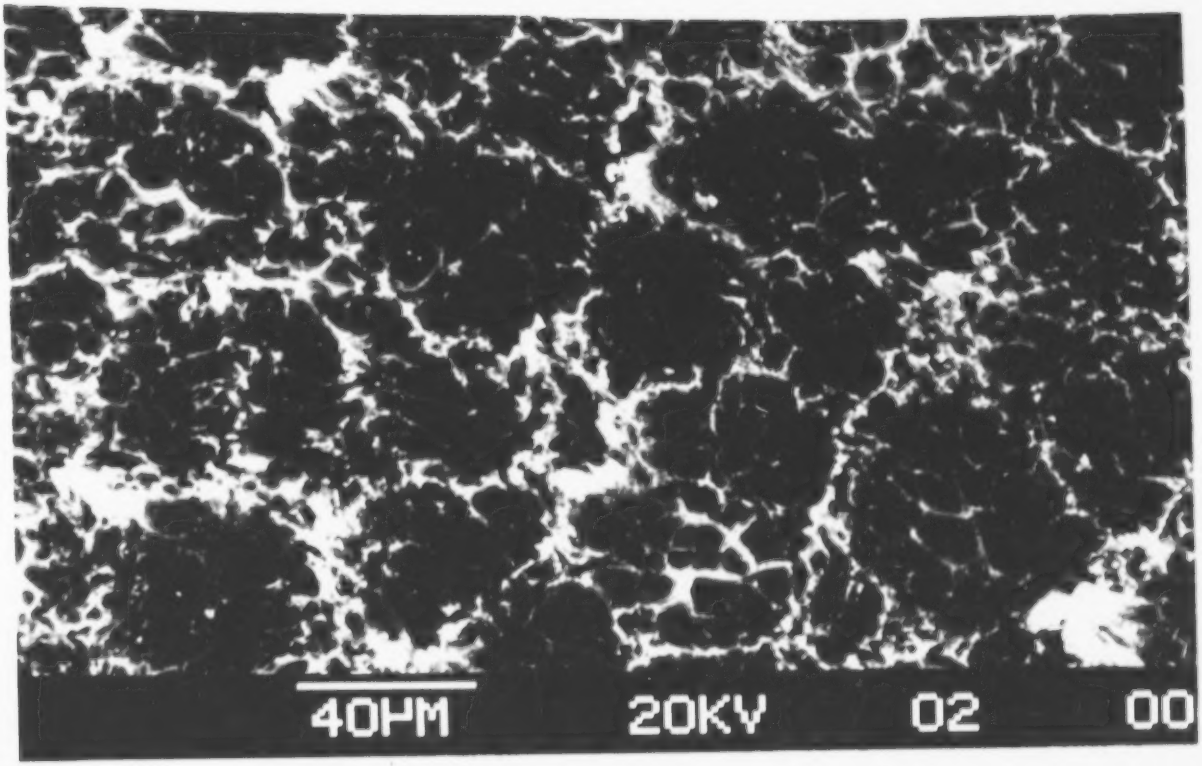


Figure 2.4 (iii) SEM Photomicrograph of a Ti Alloy Horn Tip After 15 hrs of Use and Polishing with Fine Emery Cloth.

Sonication had physically removed particles of copper from the surface of the wire. Microscopic examination of the surface did not provide any useful new information.

To determine the rate at which Cu was being eroded from the surface by sonication, pre-weighed segments of Cu wire suspended in 40 mL of water were sonicated at full power for varying time intervals. The segments were then removed, dried and weighed. The liquid phase was then evaporated to dryness on a hot plate, the resulting residue dissolved in concentrated nitric acid, and analyzed for Cu by iodimetric titration. The amount of dissolved and particulate Cu (see Table 2.2) was found to increase as a function of sonication time.

Table 2.2 Effect of Sonication on Copper Wire.

sonication time (min)	weight loss (mg)[1]	amount found (mg)[2]
0	0	0
5	4.35	4.33
10	6.05	6.11
20	11.42	11.01
40	15.96	15.85
60	19.75	19.77

[1] Refers to the difference in weight of each wire before and after sonication.

[2] Refers to the value obtained by titration.

2.3.3.3 Possible Mechanisms of Cavitational Damage

The origin and mechanisms of cavitational damage have been discussed in the literature. Lauterborn and Hentchel[189] describe cinematographic experiments for studying bubble motion during a single cycle of the driving sound field. The authors noted that the relatively low framing speeds used could not resolve details of bubble collapse during the last few nanoseconds of their lifetime.

Recent high-speed (1 million frames per second) holocinematographic studies on cavitation dynamics of a single laser-produced bubble have revealed a wealth of information about the physical aspect of bubble collapse.[190] Pictures of collapsing bubbles in bulk water reveal the existence of radiating shock waves upon collapse, whereas pictures of bubbles collapsing near extended surfaces show microjets of solvent impinging on the surface at velocities estimated to be between 100 and 200 m/sec.[191]

The experiments reported in this study show that the shock-waves and microjets produced during cavitational collapse have sufficient energy to cause morphological changes to particles suspended in water. Subsequent erosion and fragmentation lead to dramatic increases in the surface area of the solids. This physical action of ultrasound is precisely what is needed to enhance dissolution rates of solid samples. The next section describes the physical action of ultrasound on biological samples.

2.3.4 Morphoiogical Studies of Insonated Biological Sample Surfaces

The effect of sonication on the surface properties of several biological materials is presented in this section. The samples include bovine hemin, used as a model compound, a freeze-dried bovine liver reference material (used in Chapter V to assess the analytical utility of ultrasound), and cellulose, a chemically resistant polysaccharide found in plant materials.

2.3.4.1 Morphological Studies of Bovine Hemin

Hemin is a water insoluble iron-containing biological chelate. To study the effect of sonication on hemin, 50-mg portions of the powder were weighed into a 50-mL sonication vessel. Approximately 40 mL of water were added and the suspension was sonicated at full power. Samples for SEM observation were removed from the vessel at preselected time intervals. They were allowed to air dry for 2 days at room temperature, then lightly sprinkled on adhesive tape attached to an aluminum platform. Sample and platform were sputtered with gold under vacuum before mounting on the SEM.

A scanning electron micrograph of hemin before ultrasound treatment is shown in Figure 2.5(i). The material is composed of distinct crystals 60 to 100 μm on a side. After 30 minutes of sonication, typical particle dimensions were reduced to about 20 μm (Figure 2.5(ii)), and the surfaces of the crystals were roughened extensively. After 1 hr of sonication, the particles had been reduced further to about 5 μm (Figure 2.5(iii)), and individual particles showed not only surface erosion, but appeared to have undergone considerable fragmentation. The surface area had increased 100 to 1000 fold, and that the increase was a function of the number of cavitation events that occurred.

A separate experiment was done to determine whether heating hemin in boiling water would have a similar effect. A 50-mg portion of hemin was suspended in 40 mL of water and boiled for 1 hr. Figure 2.5(iv) shows a scanning electron micrograph of the crystals after this treatment. There appears to be no change in morphology or surface area. This shows that particle disintegration was not directly caused by the heating that occurred during sonication.

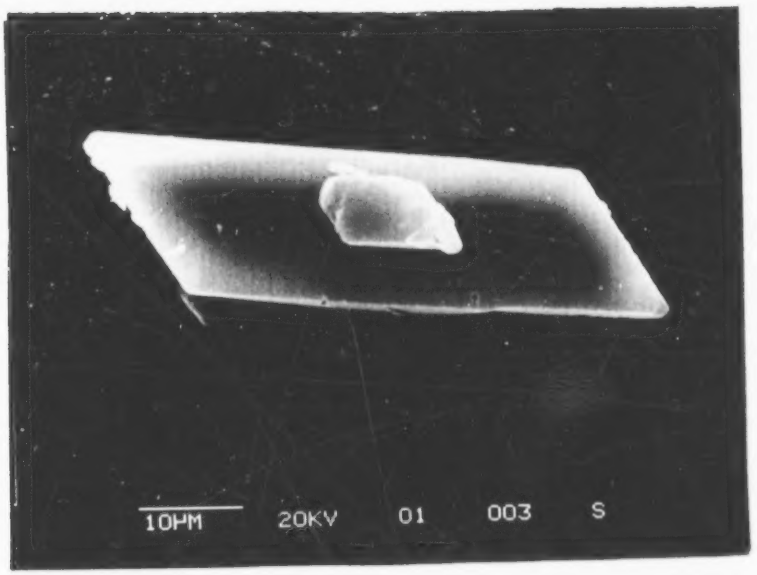


Figure 2.5(i) SEM Photomicrograph of Hemin Particles Taken Before Sonication

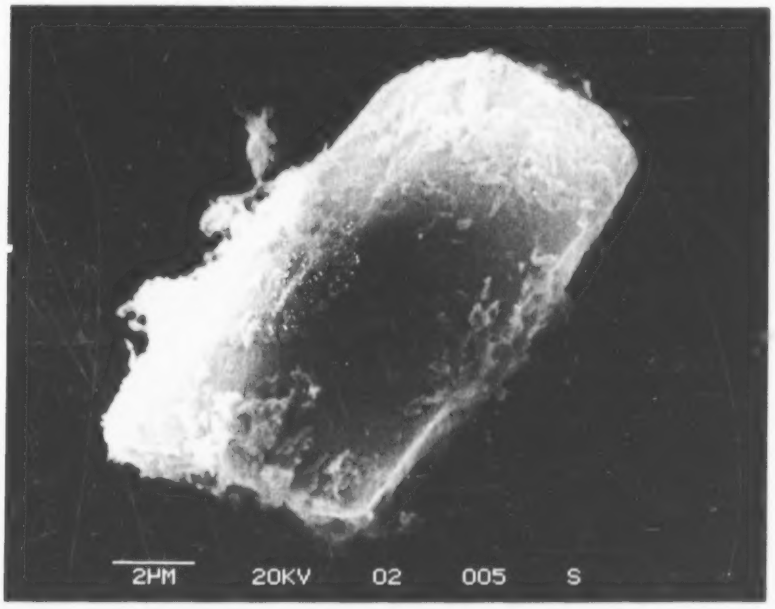


Figure 2.5(ii) SEM Photomicrograph of Hemin Particles Taken After Sonication in Water for 30 min.

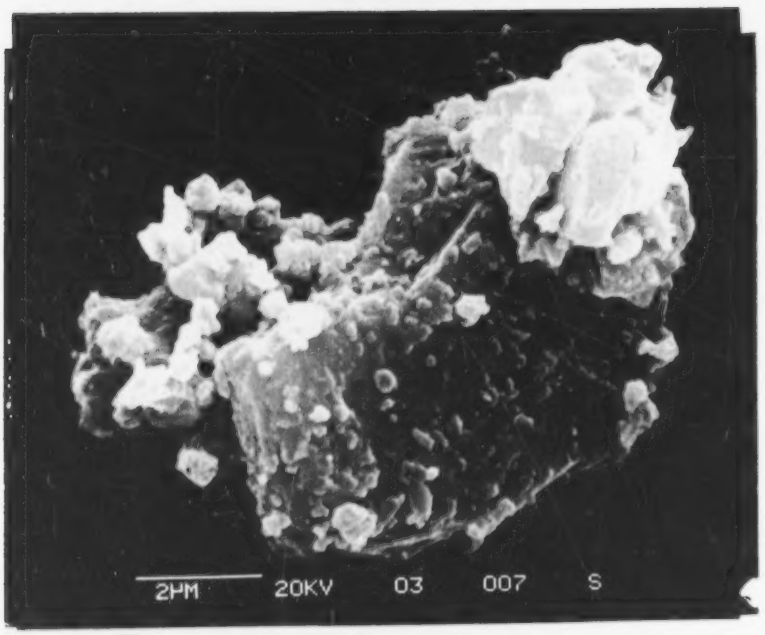


Figure 2.5(iii) SEM Photomicrograph of Hemin Particles Taken After Sonication in Water for 1 hr.

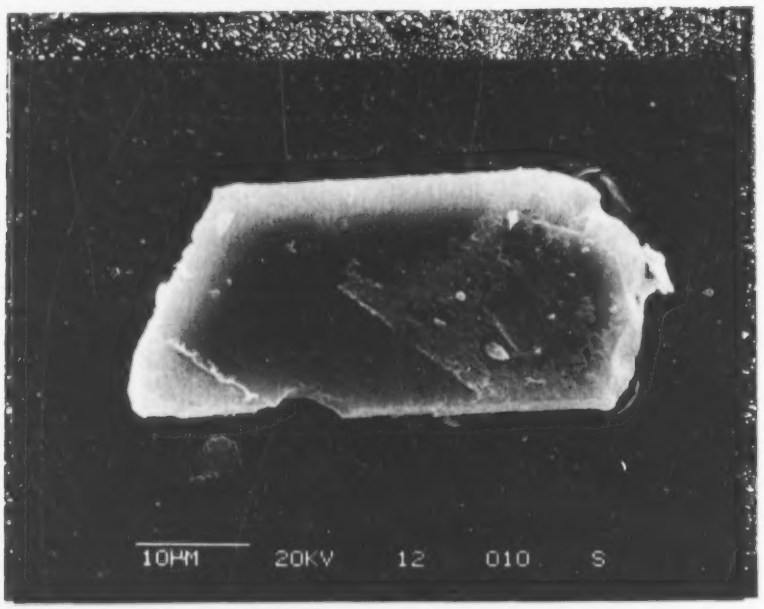


Figure 2.5(iv) SEM Photomicrograph of Hemin Particles Taken After Boiling in Water for 30 min.

The dissolution of metal-containing compounds in biological samples may be expected to occur faster when ultrasound is used rather than conventional hot-plate dissolutions because of the increase in surface area produced by ultrasound. This is explored in more detail in Chapter III. The final two sections of this chapter deal with observations of physical changes that take place when biological materials are sonicated. Bovine liver and cellulose were chosen to represent zoological and botanical sample types, respectively.

2.3.4.2 Morphological Studies of a Dried Bovine Liver Reference Material

50-mg portions of a powdered bovine liver reference material (SRM 1577a from NIST) suspended in 40 mL of water were sonicated and examined for changes in particle size and surface morphology. Figure 2.6(i) is a photomicrograph of a single fragment of liver sample before sonication. A typical particle is about 100 microns in diameter. Figure 2.6(ii) is a photomicrograph of a particle of liver sample after 30 min of sonication; Figure 2.6(iii) is a photomicrograph of a particle after 60 min of sonication. The surface appears to have changed physically because of cavitation, although not quite as extensively as hemin; this may be attributed to the fatty nature of this sample. Figure 2.6 (iv) shows the surface of a particle after boiling in water for 1 hr. As expected, no significant changes in size or morphology were observed.

2.3.4.3 Morphological Studies of Granular Cellulose

50-mg portions of granular cellulose were suspended in 40 mL of water and sonicated at full power. Samples were withdrawn at preselected time intervals and examined under the SEM microscope following drying, mounting and gold sputtering as described in section 2.3.4.1.

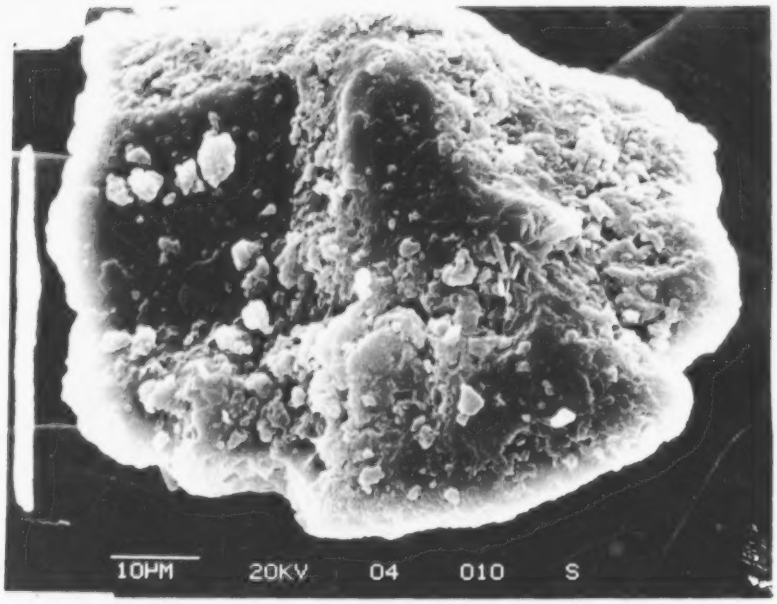


Figure 2.6(i) SEM Photomicrograph of Bovine Liver Powder Taken Before Sonication.

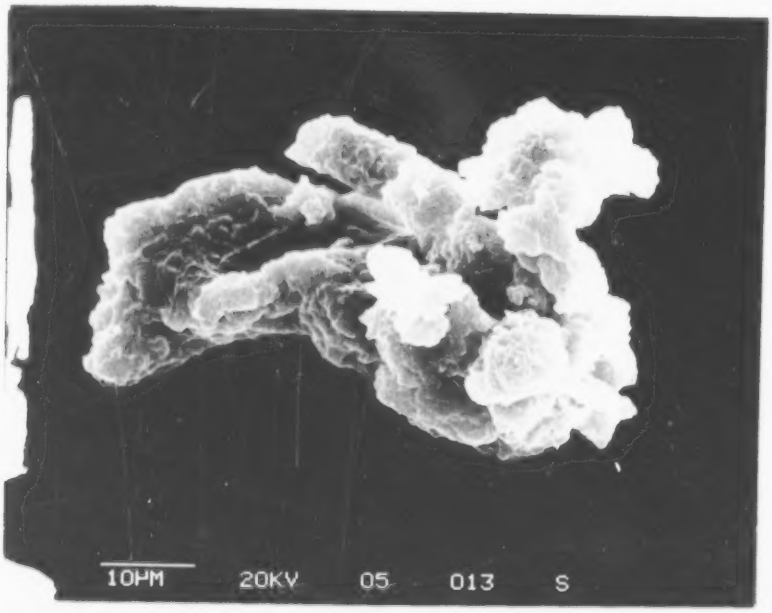


Figure 2.6(ii) SEM Photomicrograph of Bovine Liver Powder Taken After Sonication in Water for 30 min.

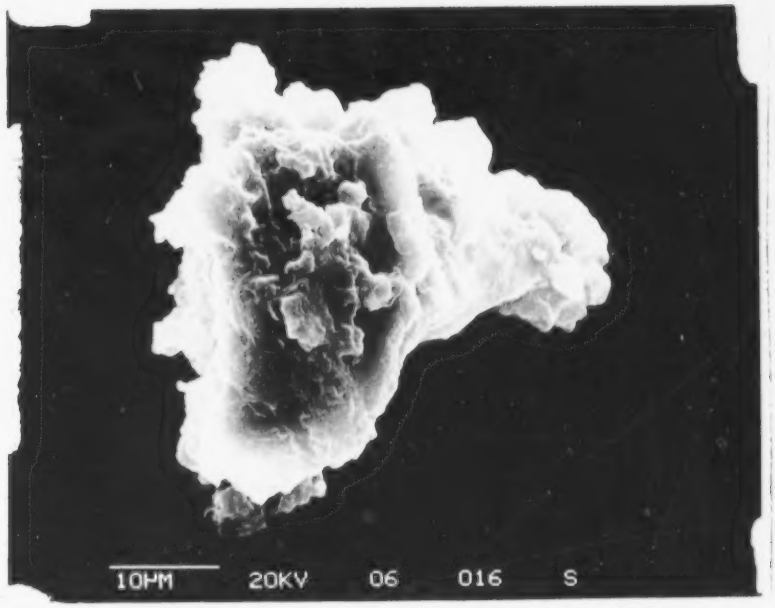


Figure 2.6(iii) SEM Photomicrograph of Bovine Liver Powder Taken After Sonication in Water for 1 hr.

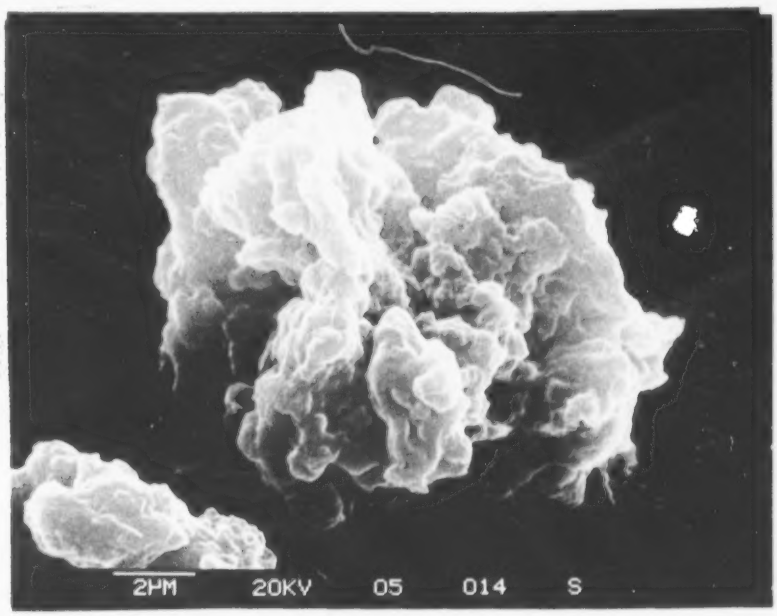


Figure 2.6(iv) SEM Photomicrograph of Bovine Liver Powder Taken After Boiling in Water for 1 hr.

Figure 2.7(i) shows the fibrous structure in a particle of sample before sonication. Figure 2.7(ii) shows a similarly sized particle after sonication for 30 minutes, and Figure 2.7(iii) shows another after 60 minutes of sonication. Figure 2.7(iv) shows a particle after boiling in water for 60 minutes. Ultrasound produced extensive erosion which increased with sonication time. The fibers were fragmented and disrupted, providing an increased solvent-accessible surface area. Samples boiled in water did not show these characteristics.

Lastly, boiling the water used for sonicating the solids studied here immediately prior to sonication to remove dissolved gases produced very different results in that no changes to the solids were observed. The reason for this is absence of cavitation when no gases are available for bubble formation. This behavior was studied in more detail; the findings are reported in Chapter III.

Figure 2.7(i) SEM Photomicrograph of Granular Cellulose Taken Before Sonication.

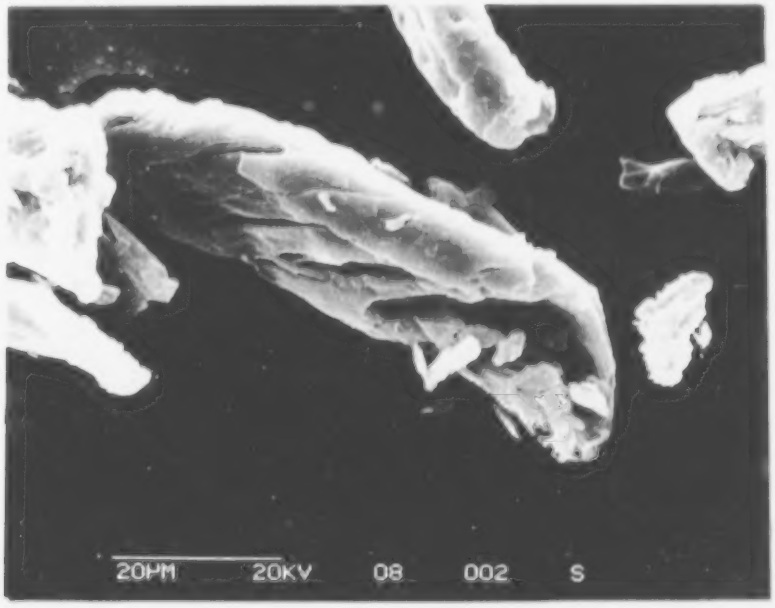


Figure 2.7(ii) SEM Photomicrograph of Granular Cellulose Taken After Sonication in Water for 30 min.

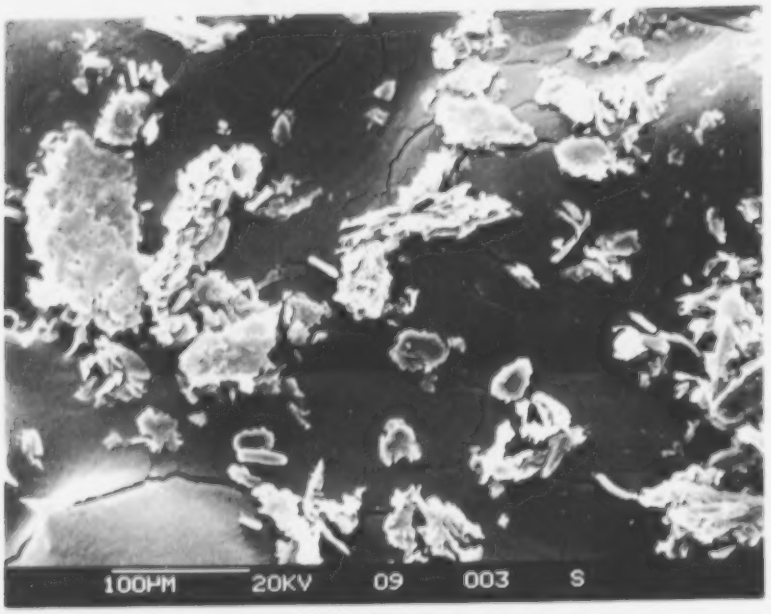


Figure 2.7(iii) SEM Photomicrograph of Granular Cellulose Taken After Sonication in Water for 1 hr.

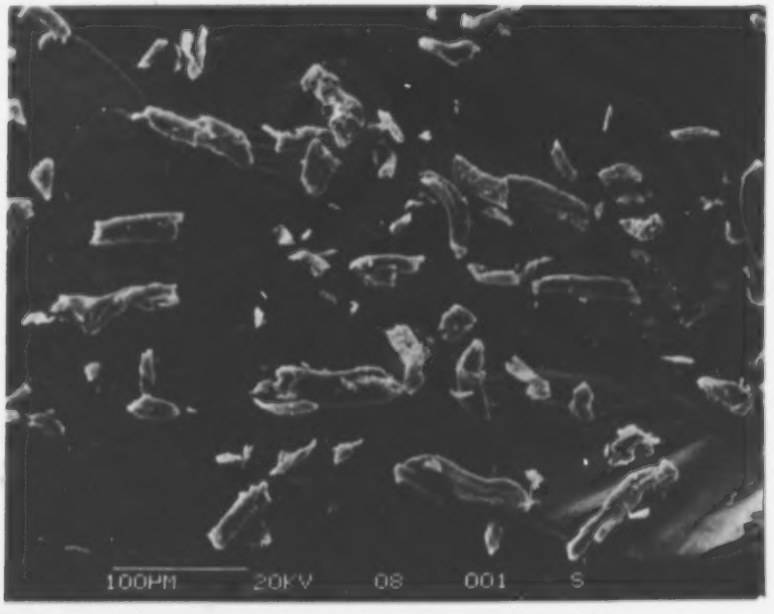


Figure 2.7(iv) SEM Photomicrograph of Granular Cellulose Taken After Boiling in Water for 1 hr.

2.4 SUMMARY

The results presented in this chapter strongly suggest that cavitation is a primary mechanism in the production of morphological changes at solid surfaces when ultrasound energy is applied to a two-phase liquid-solid system. Insonated chalk and Al foil provide evidence of localization of physical damage, and this is consistent with cavitation being the cause of surface damage which occurs upon sonication of solids suspending in a liquid. Shock waves and microjets arising from collapsing bubbles have sufficient momentum to readily erode biological materials and even metals such as copper and titanium are attacked. As mentioned before, this is important because chemical digestion of a solid in a solvent system occurs, to a large extent, on the available surface area.[192] In analytical applications where chemical digestion is required to release elements from a solid matrix into solution, physical operations such as maceration or grinding are often done prior to chemical treatment. The results presented in this chapter show that ultrasound can provide rapid increases in the surface area of suspended solids.

Lastly, equations 6 and 7 (section 2.1.1) indicate that in addition to the physical phenomena noted in this chapter, chemical reactions may be expected when ultrasound is propagated into water. The nature of these chemical processes, and the effect of dissolved gases on them, is explored in the following chapter.

CHAPTER III

STUDY OF PROCESSES THAT OCCUR ON APPLICATION OF ULTRASOUND ENERGY TO HOMOGENEOUS SINGLE PHASE AQUEOUS SYSTEMS

3.1 INTRODUCTION

The results of a preliminary study on the physical effects of 20-kHz ultrasonic sound waves on solids suspended in water were presented in Chapter II. When applied to aqueous suspensions of biological materials, ultrasound energy was found to cause pitting and erosion of the particles, thereby increasing the surface area of the sample. Evidence was presented to show that acoustic cavitation produced these effects, and thermodynamics-based cavitation equations predicting enormous local pressures caused by bubble collapse were briefly reviewed. The equations also suggest that chemical effects may occur as a result of high local cavitational temperatures. In Chapter II, it was noted that sonication of Al foil for 60 minutes resulted in significant chemical reaction with water. Normally Al reacts with H_2O to liberate hydrogen gas until formation of a passivating oxide film on the surface of the metal causes the reaction to cease. Following sonication, hydrogen evolution continued for several days, suggesting that ultrasound had not only removed the passivating oxide coat, but also formed stable chemical species in solution that were more reactive towards Al than water.

To study some of the chemical reactions involved in ultrasonic dissolutions, we first consider the behavior of the solvent water when subjected to ultrasonic irradiation, and then determine the optimum conditions for sample dissolution with the ultrasound equipment used in this study.

3.1.1 A Brief Review of Chemical Reagents Generated by Ultrasound

In 1927, Loomis[193] first reported liberation of I_2 upon sonication of aqueous solutions containing KI and CCl_4; the reaction was attributed to cavitation. Numerous subsequent reports of chemical reactions initiated by ultrasound are found in the literature. They include oxidation of H_2S to form colloidal sulfur, decomposition of compounds such as NI_3, and depolymerization.[144, 194] All these reports suggest that the driving force behind the chemical effects is cavitation.

3.1.2 Acoustic Cavitation

Cavitation in water was first described by Burnaby in 1895,[195] who produced it by the mechanical motion of a rotating blade. In 1917 Lord Rayleigh[179] first formulated a mathematical equation describing cavitation.

The adiabatic compression of gas and/or vapors inside a cavitating bubble generates heat. Thermodynamic calculations (equation 6)[196] and spectroscopic studies on the sonoluminescence of silicone oil (polydimethylsiloxane) by Suslick[197] indicate that temperatures of around 5000 K are produced. These extreme conditions are sufficient to break water molecules into free radicals:

$$H_2O \Rightarrow \cdot OH + \cdot H$$

These radicals are short-lived and react to form a variety of products, especially H_2 and H_2O_2:

$$\cdot H + \cdot H \Rightarrow H_2$$

$$2\ \cdot OH \Rightarrow H_2O_2$$

In the presence of oxygen, additional hydrogen peroxide is formed when the hydrogen radicals combine with O_2 :

$$\cdot H + O_2 \Rightarrow \cdot HO_2$$

$$\cdot HO_2 + \cdot HO_2 \Rightarrow H_2O_2 + O_2$$

The existence of hydroxyl free radicals was confirmed using spin trap e.s.r. techniques,[198] and that of H_2O_2 by colorimetric methods.[199]

Substances in a solution being sonicated often may react with $\cdot OH$ or H_2O_2. These may be dissolved solutes, or particles in suspension. Hydroxyl free radicals and hydrogen peroxide are often especially effective in oxidising organic compounds. Internal generation of a reagent for sample dissolution is desirable because it reduces the likelihood of contamination from external reagents, especially for biological samples.

This chapter explores cavitation and sonication, with the aim of finding ways of maximizing the yield of $\cdot OH$ and H_2O_2.

3.2 EXPERIMENTAL

3.2.1 Reagents and Solutions

Ultrapure water (18.3 M.Ω.cm resistivity) was prepared by distillation, followed by passage through a Barnstead D4751 NANOpure@ Ultrafiltration System (Dubuque, Iowa, USA). H_2O_2, $NaNO_3$, Na_2SO_4, NaCl, HNO_3, HCl, HF, H_2SO_4, KI, KNO_3, CH_3COOH, $NaOOCCH_3$, and $NH_4Mo_2O_7$ (BDH Chemicals, Toronto, Canada) were all reagent grade. Standard H_2O_2 solutions were prepared by dilution of the 30%v/v stock solution. Compressed oxygen, nitrogen, and argon (Linde, Union Carbide Canada Ltd.) were used without further purification.

3.2.2 Equipment

3.2.2.1 Sonication Apparatus

The ultrasound horn system described in Chapter II was used with minor modifications. Additional holes were drilled in the back of the safety cabinet for tubing to introduce gases to solutions as a fine stream of bubbles from a tapered glass capillary tip. The arrangement is shown schematically in Figure 3.1. A gas flow rate of 50 mL per minute was found optimum in most cases.

3.2.2.2 UV-visible Spectrophotometry of Hydrogen Peroxide

Spectrophotometric determinations of hydrogen peroxide were carried out indirectly by measurement of I_2 formation from iodide using a photodiode array spectrophotometer (Hewlett Packard model 8451A).

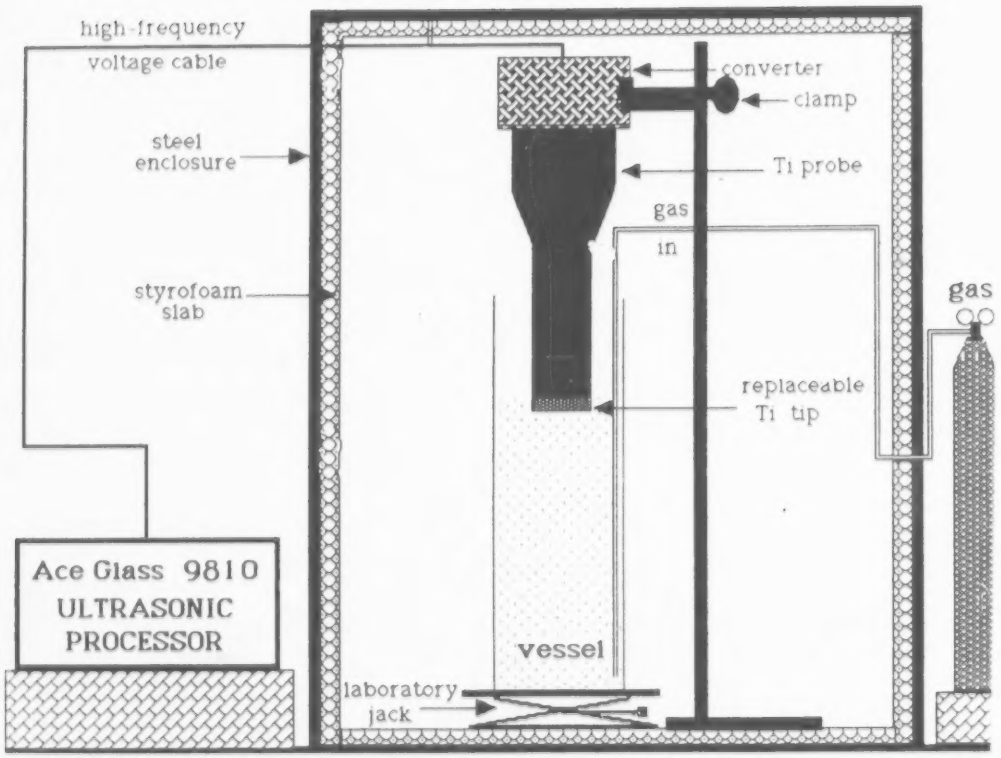


Figure 3.1 Schematic Diagram of a Sonochemical Reaction Assembly for the Study of Sonication Processes in a Homogeneous Single-Phase Aqueous System.

3.2.3 Methods

For experiments involving gases, the vessels were immediately covered with parafilm to prevent re-diffusion of atmospheric gases back into solution.

3.2.3.1 Determination of H_2O_2

3.2.3.1.1 Iodometric Titration

The amount of hydrogen peroxide produced from sonolysis of water was determined by addition of excess KI and titration of the iodine formed with thiosulfate. The procedure used was as follows. To 80 mL of sample was added 2 mL of a 1 to 3 H_2SO_4 : H_2O solution, followed by addition of 0.2 g KI and 2 drops of saturated ammonium molybdate solution. The liberated iodine was then titrated with 0.001M $Na_2S_2O_3$ to a starch end-point.

3.2.3.1.2 Spectrophotometric Measurement

In later work, the time-consuming titration with thiosulfate was omitted; instead, the yellow brown color of the triiodide ion was measured spectrophotometrically using an HP8451A UV-vis spectrophotometer. A blank was prepared by addition of all reagents to 80 mL of water, but without sonication.

For standardization, solutions of H_2O_2 ranging from 0 to 50 micromolar were prepared by pipetting appropriate quantities of a 100 μM stock solution. After a 10-minute delay for color development, the solutions were transferred to standard 1-cm path length quartz cells for measurement.

To monitor the analytical peak at 352 nm, the spectrometer was programmed to scan between 290 and 490 nm. Absorbances at λ_{max} were obtained via a peak search routine provided by the operating software of the instrument.

3.2.3.1.3 Modification of Kingzet Method for Peroxide Determination

Figure 3.2 shows the spectrum of a solution prepared by treating a 20 μM peroxide standard with excess potassium iodide in dilute sulfuric acid, then allowing the color to develop for 5 minutes. An in-built software program to calculate the first derivative plot of the spectrum was used to locate two triodide maxima at 292 nm and 352 nm. The molar absorptivities at these two wavelengths were found to be $\varepsilon_{\lambda 292}$=10,050 $l.mol^{-1}.cm^{-1}$ and $\varepsilon_{\lambda 352}$=10,500 $l.mol^{-1}.cm^{-1}$. The peak at 352 nm was chosen for all subsequent peroxide determinations.

Historically,[193] the liberation of iodine by sonicated solutions containing KI has been used as a model system to demonstrate the activation of chemical reactions by ultrasound. The effect of time on iodine production is usually ignored when estimates of peroxide in sonicated water are made using this reaction. Time was investigated here as one of several factors on which information was needed if the process was to be useful analytically.

The oxidation of iodide by peroxide is slow. Kingzett used ammonium molybdate as a catalyst to speed up the reaction.[200] Figure 3.3 shows the effect of molybdate on the reaction between an 8 μM solution of peroxide and acidic KI. The trend observed is an initial linear increase of absorbance with reaction time, followed by a fall-off as the peroxide is consumed. With the addition of molybdate the increase in absorbance is sharper, as expected. On the basis of these curves, it can be concluded that the rate of the peroxide-iodide reaction must be taken into account when considering the rate of oxidant production. Accordingly, in the presence of molybdate, it is necessary to make measurements only after reaction times of 10 minutes or more. In the absence of molybdate, the time must be increased to 25 minutes or more, where absorbance changes with time are small.

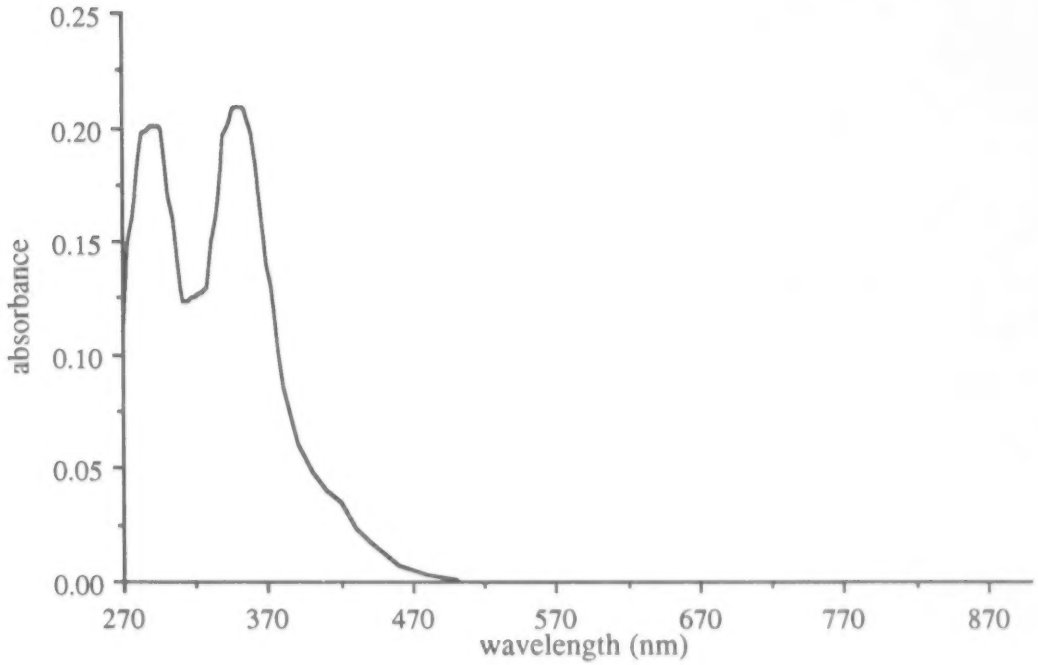


Figure 3.2 Absorption Spectrum of I_3^- Ion. A Hewlett-Packard photodiode array uv-vis spectrophotometer was used to acquire spectrum; color was developed using the Kingzett procedure on a solution containing 20 μM H_2O_2.

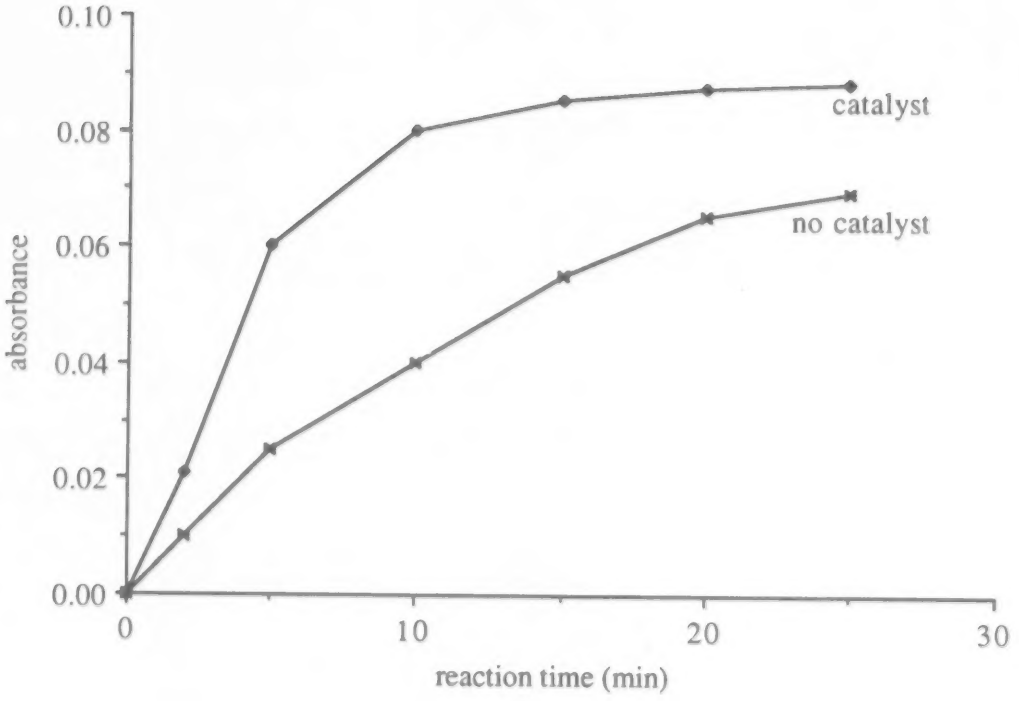


Figure 3.3 Effect of Ammonium Molybdate Catalyst on Rate of Peroxide-Iodide Reaction. n=3; uncertainty at $\pm 1\sigma = 8$ %.

Also it should be noted that a true steady state is not reached in these measurements. This may be due to oxidation of some iodide ion by dissolved oxygen. Photochemical oxidation of iodide may also cause problems in these measurements; to reduce this effect, exposure of the solutions to room light was kept to a minimum.

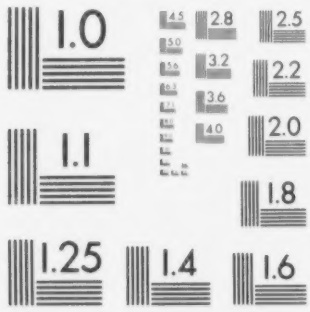
The relationship between I_3^- absorbance and concentration of added H_2O_2 using the procedure described above is shown in Figure 3.4. These values were used to construct the calibration plot shown in Figure 3.5. The Beer-Lambert law is observed in the concentration range of interest. From the slope of this plot, the molar absorptivity of the triiodide ion at 352 nm was found to be 15,700 L. mol^{-1}. cm^{-1}.

Figure 3.6 shows a spectrum of water in equilibrium with air that was sonicated for 30 minutes. The spectrum was taken 10 minutes after KI and molybdate were added. The generation of oxidant by ultrasound is confirmed, and, more importantly, there appear to be no spectral interferences at the wavelength selected for analysis.

To assess the reliability and accuracy of the spectrophotometric method for peroxide determination, two sets of five samples of pure water in equilibrium with air were sonicated at full power for 25 minutes. Spectroscopic measurements of the first set by the procedure described above gave a value equivalent to 10±2μM peroxide. The Kingzet titration on the second set gave a value of 11±3μM. The results obtained by the two methods are not statistically different.

To summarize, time for the peroxide-iodide reaction to proceed was found to be very important in these measurements.

PM-1 3½"x4" PHOTOGRAPHIC MICROCOPY TARGET
NBS 1010a ANSI/ISO #2 EQUIVALENT



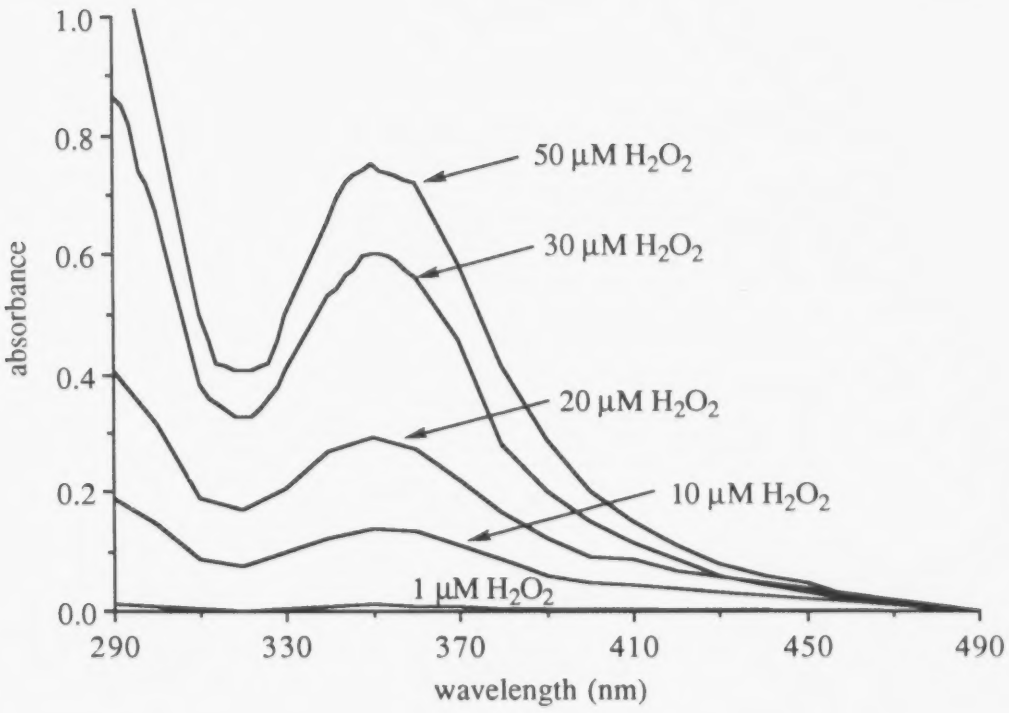


Figure 3.4 Absorbance as a Function of Wavelength for I_3^- Produced from Hydrogen Peroxide Standards. Color development was for 10 min.

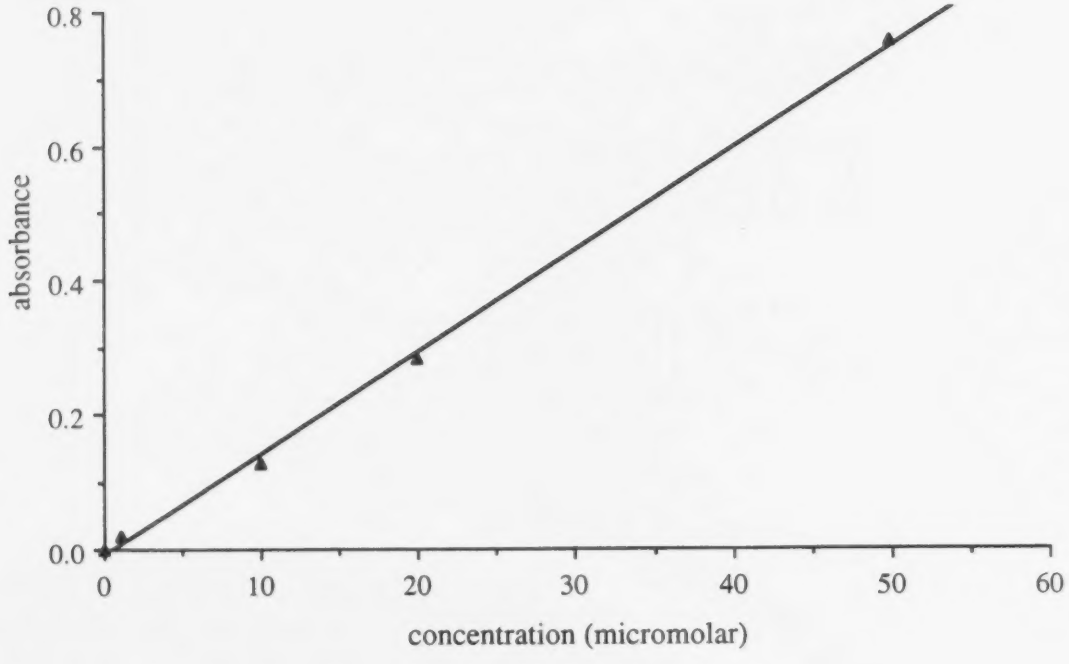


Figure 3.5 Calibration Plot for Determination of Hydrogen Peroxide. Least squares calculation gives equation of line as y = 0.0157x - 0.0012.

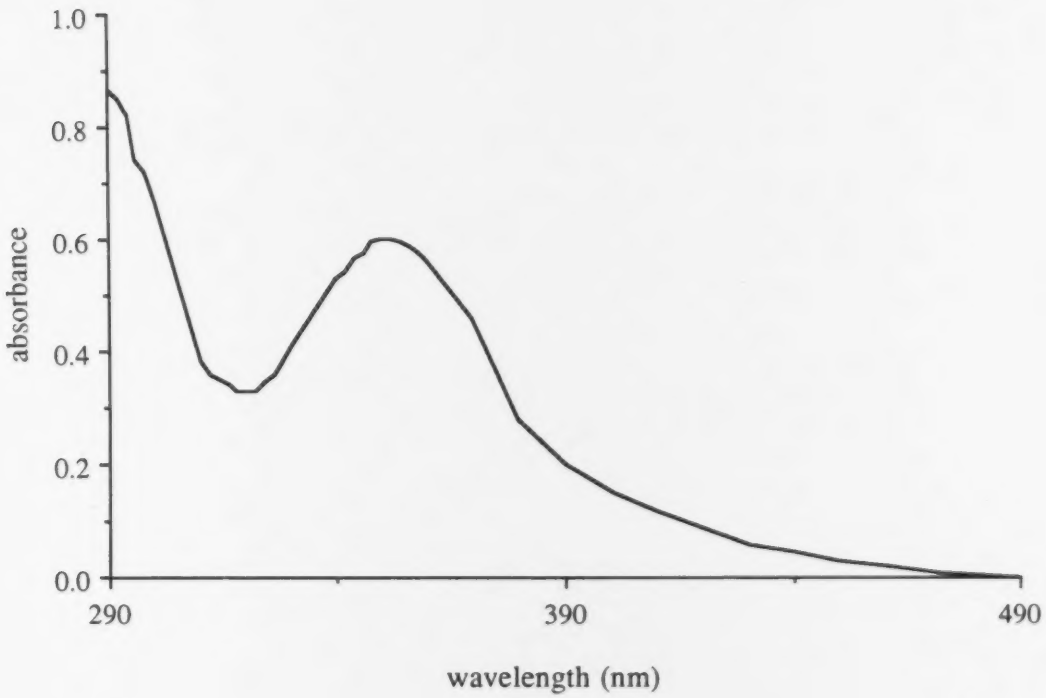


Figure 3.6 Absorption Spectrum of a Water Sample (after development of color) that was Sonicated for 30 minutes. The spectrum was taken 10 minutes after reagents were added.

Sonochemical yields of peroxide reported in the literature may be lower than the true values, especially for measurements carried out by direct sonication of KI solutions without due consideration of the effect of time before measurement or use of catalyst.

3.2.3.2 Polarographic Determination of O_2

Dissolved oxygen in sonicated solutions was measured with a PAR174A Polarographic Analyzer (Princeton Applied Research) using a dropping mercury electrode (DME) and a saturated calomel reference electrode (SCE). Both classical direct current and differential pulse polarography were used, the former as a check for interferences, and the latter for quantitative determinations. The DME set-up was adapted from a previously described procedure for dissolved oxygen.[201] All samples were buffered at pH 4.7 by addition of deaerated $NaCOOCH_3/CH_3COOH$ buffer solution to the sonicated solutions. Solid KNO_3 was also added to make the solution 0.1 M in KNO_3 as supporting electrolyte. In addition, 3 drops of a 1% solution of Triton X-100 were added as maximum suppressor for the oxygen waves. Both DC and DPP current-voltage curves were recorded on a chart recorder; the operating conditions are shown in Table 3.1.

3.3 RESULTS AND DISCUSSION

The yield of radicals and hydrogen peroxide in sonicated water depends on many factors. Among the more important are temperature, ultrasound frequency and intensity.[144]

Table 3.1 Operating Conditions for the PAR 174A Polarographic Analyser.

Parameter	Direct Current	Differential Pulse
Initial Potential	0.200V	0.200V
Potential Range	3.00V	3.00V
Scan Rate	5 mV/s	2 mV/s
Modulation Amplitude	25 mV	25 mV
Current Range	10 μA	50 μA
Drop Time	2 sec	1 sec

Most of the investigations reported in the literature were carried out using ultrasound baths; only limited information is available on ultrasound horns. Furthermore, differences exist among individual designs in terms of performance. The results reported in this study were obtained using a Sonics and Materials horn marketed by Ace Glass Inc. (Model 9810).

Throughout this study, we shall refer to peroxide formation and peroxide levels generated by sonication. This is done for convenience in plotting; the actual species generated during sonication were not identified. What we know is that these species react with I^- to form I_2. Most, perhaps all, may be H_2O_2, but this has not been proven. It is likely that at least part of transient species such as ·OH and O_2^- may react directly with iodide without the formation of peroxide as an intermediate.

3.3.1 Effect of Sonication Time on Ultrasound-Produced Hydrogen Peroxide

To study the rate of production of H_2O_2, 40-mL portions of water were sonicated at full power and analyzed for peroxide by iodimetric titration. The results of a series of measurements at differing sonication times are shown in Figure 3.7. The amounts of peroxide formed increase rapidly with time during the first 10 min of sonication, then more slowly to a concentration of approximately 13 μM after 25 min.

It can be concluded from these results that sonication of water produces substances capable of oxidising iodide to iodine. This could explain the observed reactivity of Al in water for many hours after application of 600W ultrasonic energy (see section 3.1).

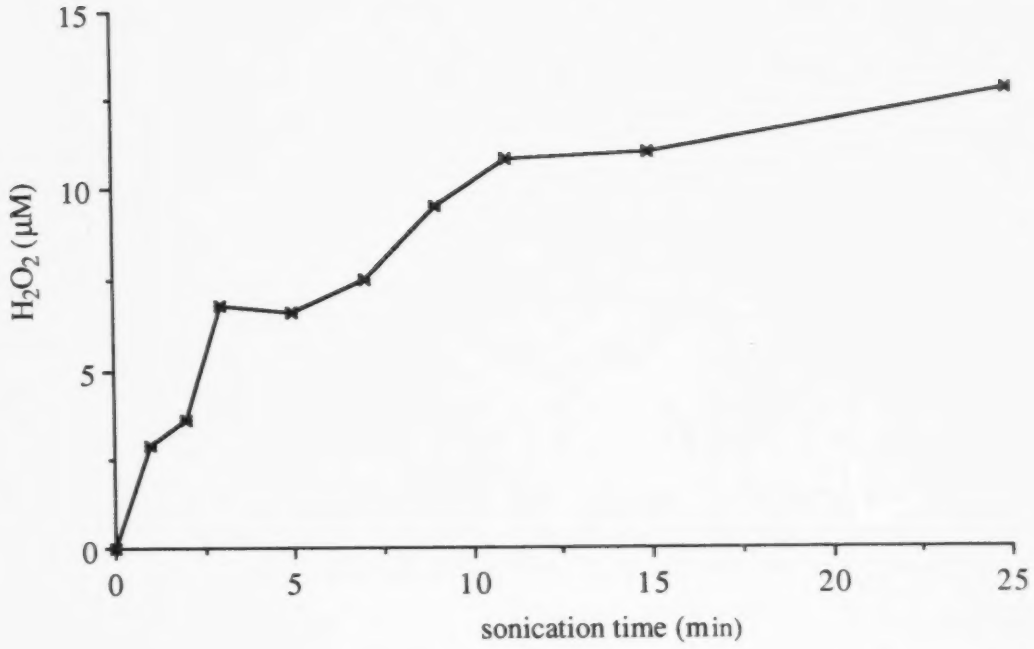


Figure 3.7 Levels of Oxidant Produced (as hydrogen peroxide) from Water in Equilibrium with Air as a Function of Sonication Time. n=8; uncertainty at $\pm 1\sigma$ = 8 %.

The initial increase in peroxide formation as a function of sonication time can be attributed to the increasing number of cavitation events. The leveling of the plot after about 10 min also shows that the generation of oxidizing species falls off with time. To determine the reason for this several conditions were studied. These included probe position, ultrasonic power, the composition and concentration of dissolved gases, temperature, and solution acidity.

3.3.2 Effect of Probe Location in the Sonication Vessel

According to equation 4, ultrasound intensity is greatest immediately below the tip of the probe, where attenuation is zero, then drops off as a function of the medium in which the probe is immersed. The optimum position for maximum sonochemical yield of peroxide as measured iodometrically was determined by sonicating 40 mL of water for 15 minutes at full power at different probe heights from the cell bottom. The results of the iodometric titrations plotted against distance between the probe and the cell bottom are shown in Figure 3.8. There is an increase in the level of peroxide with distance up to 4 cm, after which a sharp drop is seen. Oxidant levels increase again to a peak at a distance of 8 cm, when they again drop. This saw-tooth pattern is repeated at approximately 4-cm intervals. These repetitive cycles cannot be explained on the basis of ultrasound attenuation.

According to equation 2, the wavelength of 20 kHz ultrasound passing through water at a speed of 1500m/s is approximately 7.5 cm. The observed repetition in the results occurs at approximately multiples of half a wavelength ($n\lambda/2$) of the ultrasound wave. This seems to indicate that a standing wave is established inside the sonication medium. Constructive interference of the sound waves as they bounce off the bottom of the vessel occurs if the standing wave condition is satisfied.

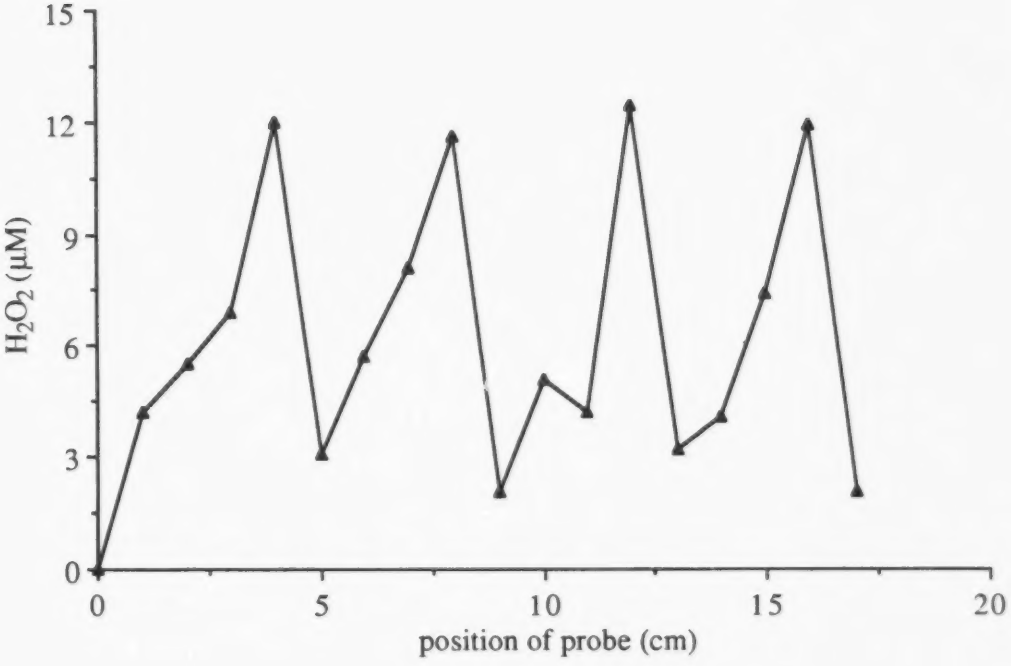


Figure 3.8 Effect of Position of Probe Tip Relative to Sonication Vessel Bottom, on Oxidant Levels in Sonicated Water. Sonication time and power were held constant (15 min and 600 W respectively). n=3; uncertainty at $\pm 1\sigma = 15$ %.

If reflection from the cell bottom is 100% efficient and attenuation is negligible, cavitation bubbles should experience double the intensity delivered by the probe under standing wave conditions, i.e. when the probe is 4, 8, 12, 16.... cm above the bottom of the vessel.

The results suggest that attenuation of the sound waves by the water column is small because the intensities at successive $\lambda/2$ points in the plot are almost constant. This is consistent with the fact that values for the attenuation coefficient α in water at 20 kHz[202] are approximately 9.9 X 10^{-8} cm^{-1}; if the initial intensity I_o is taken to be 600W, then the intensity would drop by only 1 mW (0.2 %) at a depth of 16 cm.

Consequently, all sonications following this study were performed with the probe tip located either 4 or 8 cm above the cell bottom.

3.3.3 Effect of Ultrasound Power

The strength of the ultrasound field experienced by cavitation bubbles is directly proportional to the amplitude of vibration of the probe. Operation of the Sonics ultrasound unit at the maximum power of 600 W gives an amplitude at the tip of 60 μm. By operating the unit at 50% power, the amplitude of the tip motion is expected to be reduced by half.

To study the effect of applied power on the yield of peroxide, two sets of six 40-mL portions of water in equilibrium with air were sonicated at a probe to vessel bottom distance of 4 cm. One set was sonicated at 100% power (600 W), and the other at 50% (300 W). Individual samples were sonicated for 10, 20, 30, 60, 120 or 180 min, and the peroxide produced was determined spectrophotometrically.

Plots of results for the two sets of experiments are shown in Figure 3.9.

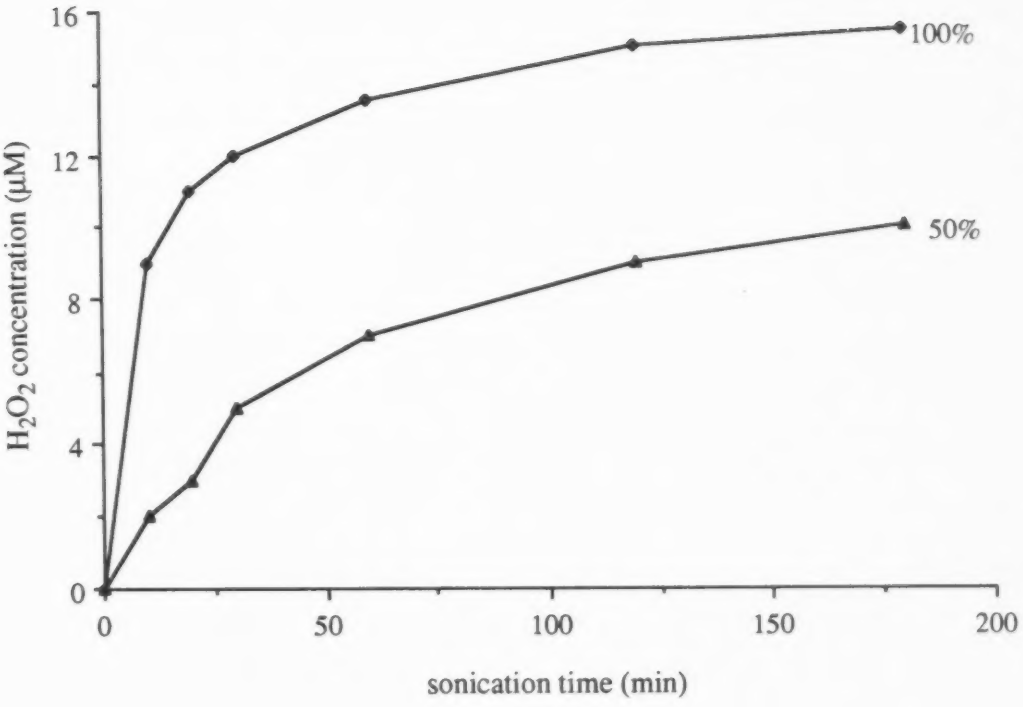


Figure 3.9 Effect of Ultrasound Power on Oxidant Levels in Sonicated Water Samples, as Measured by Iodide Conversion to I_3^- (results reported in terms of H_2O_2 concentration based on calibration plot). n=3; uncertainties at $\pm 1\sigma$ = 16%

Trends for both power levels as a function of sonication time are similar, but sonication at 100% power clearly produces higher concentrations of oxidising species than does 50% power, especially at sonication times less than 30 min. In terms of cavitation theory, it is not only the number of cavitation events that influence peroxide formation, but also the energy involved in the collapse of these bubbles. Bubble collapse is less violent at reduced power; therefore localized cavitation temperatures are reduced, and peroxide levels are lower.

3.3.4 Effect of Cavitation Gas on Generation of Oxidizing Species

An important factor affecting cavitation is the presence in the solution being sonicated of dissolved gases. This part of the study investigates the effect of type of gas present in water on the sonolysis process. For cavitation to be of most assistance in chemical digestion of sample matrices, oxidants such as free radicals and hydrogen peroxide should be produced in significant quantities in as short a time as possible.

3.3.4.1 Cavitation in the Presence/Absence of Dissolved Gases

According to Burnaby's definition,[195] if sonication is performed in water that is free of particulate matter and dissolved gases, cavitation will only occur if the ultrasound power has sufficient force to physically break hydrogen bonds between water molecules during rarefaction. In order to overcome the cohesive intermolecular forces that bind water molecules together, pressure changes in the rarefaction stage of ultrasound must exceed the theoretical tensile strength of water (1500 atm).[203]

Several experiments were conducted to test the effects of dissolved gases. Deaerated water was prepared by boiling 1000 mL NANOpure@ filtered water for 1 hr, then covering tightly with parafilm to seal out atmospheric gases while

cooling. Approximately 40-mL portions of the boiled water were sonicated immediately at 600 W and oxidant production measured spectrophotometrically. Unboiled NANOpure@ filtered water samples were also sonicated in order to compare the effect of dissolved air. The results, see Figure 3.10, show that removal of most of the dissolved gases from water by boiling prior to sonication leads to little or no cavitation by 600 W ultrasound energy at 20 kHz. Clearly the pressure differences exerted at this power level do not provide enough energy to overcome the intermolecular forces that bind water molecules together. It can be concluded that in order to generate oxidizing species from water with the ultrasound probe power and frequency used in this work, dissolved gases must be present.

3.3.4.2 A Sonochemical Model For Gaseous Cavitation

We have shown that in the presence of dissolved gases the yield of peroxide as a function of sonication time at constant power, frequency, and pressure increases sharply initially, then begins to level off (Figure 3.9). The μM concentration of oxidants produced is too low, however, to be effective for most analytical dissolutions. For ultrasound-produced oxidants to be useful for chemical digestion of biological samples, for example, it is desirable for them to be generated rapidly and in high concentration.

To obtain higher concentrations it is necessary to determine the cause of the levelling observed at longer sonication times. Once this is established, ways of increasing the rate of production can then be considered.

To better understand oxidant production (as ·OH or H_2O_2) it is necessary to consider the effect of dissolved gases on cavitation processes in a more fundamental way. This requires consideration of the physics of a gas bubble in water in an ultrasound field.

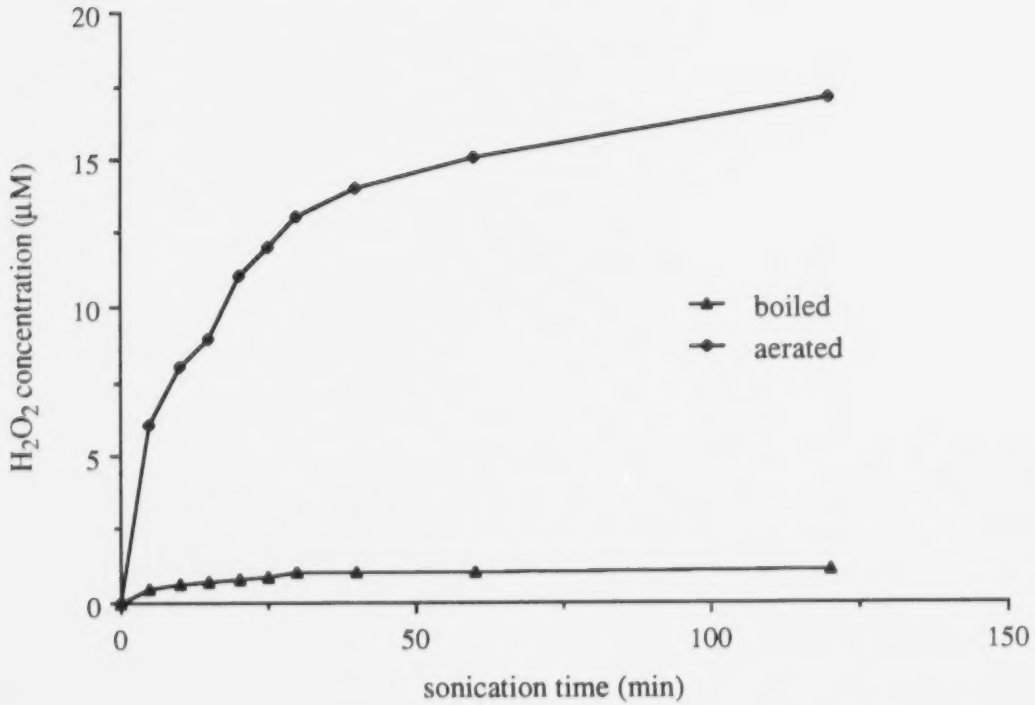


Figure 3.10 Effect of Presence or Absence of Gas on the Sonochemical Yield of Hydrogen Peroxide. n=5; uncertainties at $\pm 1\sigma = 10\%$.

Suppose that a single gas bubble of radius R in water is subjected to pressure waves whose time dependence is described by equation (1). The bubble responds to the applied pressure by oscillating in size with the field. Both internal and external environments affect the formation and collapse of the bubble. The immediate external environment is characterized by the nature of the solvent and solutes that are dissolved in it. For a bubble in pure water, the external environment is water of viscosity η, density ρ, surface tension σ, temperature T, and pressure P_o. P_o is the sum of the ambient pressure in the fluid P_h and the pressure exerted by the sound waves P_t. The internal environment is characterized by the nature of the gas and/or vapor inside the bubble.

Under the influence of the oscillating acoustic field described by equation 1, the surface of the bubble oscillates. The Rayleigh-Plesset equation:[204]

$$R\ddot{R} + \frac{3}{2}\dot{R}^2 - \frac{1}{\rho}\left[\left(P_h+\frac{2\sigma}{R_e}\right)\left(\frac{R_e}{R}\right)^{3K} - \frac{2\sigma}{R} - 4\eta\frac{\dot{R}}{R} - (P_h + 2P_A\sin 2\pi ft)\right] = 0 \quad \text{.....(8)}$$

describes the acceleration $\ddot{R}$ and velocity $\dot{R}$ with which the bubble surface moves. R_e is the equilibrium bubble radius and K is the polytropic index of the gas inside the bubble. K varies between unity and γ (defined as the ratio C_p/C_v), the limits for adiabatic and isothermal conditions, respectively.

On the assumption that bubble collapse occurs under adiabatic conditions, numerical integration of equation 8 leads to the following (see chapter II):

$$P_{max}=P\left[\frac{P_m(\gamma-1)}{P}\right]^{\gamma/\gamma-1} \quad \text{.....(6)}$$

$$T_{max}=T_o P_m \frac{(\gamma-1)}{P} \quad \text{.....(7)}$$

Equation 6 relates the maximum pressure within the bubble P_{max} to the pressure before collapse P_m, the vapor pressure of the solvent P, and the specific heat ratio γ. Equation 7 describes cavitation dynamics in terms of temperature, where T_o is the ambient temperature. Even if a bubble does not undergo complete transient collapse, extremely high temperatures and pressures are developed within it as it oscillates.

Perhaps the most significant quantity for this discussion is the specific heat ratio. As C_p approaches C_v, the specific ratio approaches unity, and according to equations 6 and 7, the maximum cavitation temperature and pressure will be low. It was noted in Chapter II that low cavitation pressures affect *sonomechanical* effects such as microjet impact and shock-wave damage when solids are suspended in a liquid. The cavitating bubble model predicts, in a similar fashion, that low cavitation temperatures are likely to affect *sonochemical* effects since the yield of free radicals depends on the cavitation temperature. According to this model, bubbles filled with monoatomic gases such as Ar are likely to show greater sonochemical effects than polyatomic gases because they have larger specific heat ratios (γ_{Ar}=1.67; γ_{oxygen}=1.39). By the same token, bubbles containing a gas of low thermal conductivity will show greater yields of free radicals and peroxide because high temperatures can be maintained for longer periods of time within such gases. Thus among the noble gases, Ar (κ=0.0173 $W m^{-1}K^{-1}$) is expected to show greater yields when compared to He (κ=0.143$Wm^{-1}K^{-1}$), even though they both have the same specific heat ratio. A summary of the thermal properties[205] of several laboratory gases is presented in Table 3.2.

Table 3.2 Summary of Thermal Properties of Several Laboratory Gases. κ is the thermal conductivity in $Wm^{-1}K^{-1}$, and γ is the specific heat ratio, C_p/C_v.

Gas	γ	κ
Carbon monoxide	1.43	0.0272
Argon	1.67	0.0173
Neon	1.67	0.0472
Helium	1.67	0.1430
Oxygen	1.39	0.0164
Nitrogen	1.40	0.0252

3.3.4.3 Evaluation of Nitrogen and Argon as Cavitation Gases

From the above discussion, it appeared worthwhile to determine whether peroxide levels could be enhanced by appropriate selection of cavitation gas. Of the gases listed in Table 3.2, Ar and N_2 should represent the extremes in terms of choice of cavitation gas.

To test this, 40-mL portions of water in sonication vessels were saturated with either Ar or N_2 by bubbling the gas for 10 minutes prior to and during sonication. The results of analyses of these solutions for oxidizing species by iodide oxidation as before are shown in Figure 3.11. As predicted by the model, the Ar saturated solutions generated more oxidants than those containing nitrogen. Nitrogen is such a poor cavitation gas, in fact, that oxidant levels are close to what was observed in water boiled to remove dissolved gases. Since the results using nitrogen are very low compared to those with air (Figure 3.10), which is 79% nitrogen, clearly the presence of oxygen in dissolved air plays a major role in the production of iodide-oxidizing species. Therefore the behavior of oxygen was investigated next.

3.3.4.4 Evaluation of Oxygen as Cavitation Gas

The objective of this study was to determine the extent to which the use of dissolved oxygen (DO) as cavitation gas affects the sonochemical yield of oxidizing species. Even though the values of thermal conductivity and specific heat ratio for Ar indicate that it would be more effective as a cavitation gas than O_2, the fact that O_2 itself is a good oxidant may counterbalance the effect.

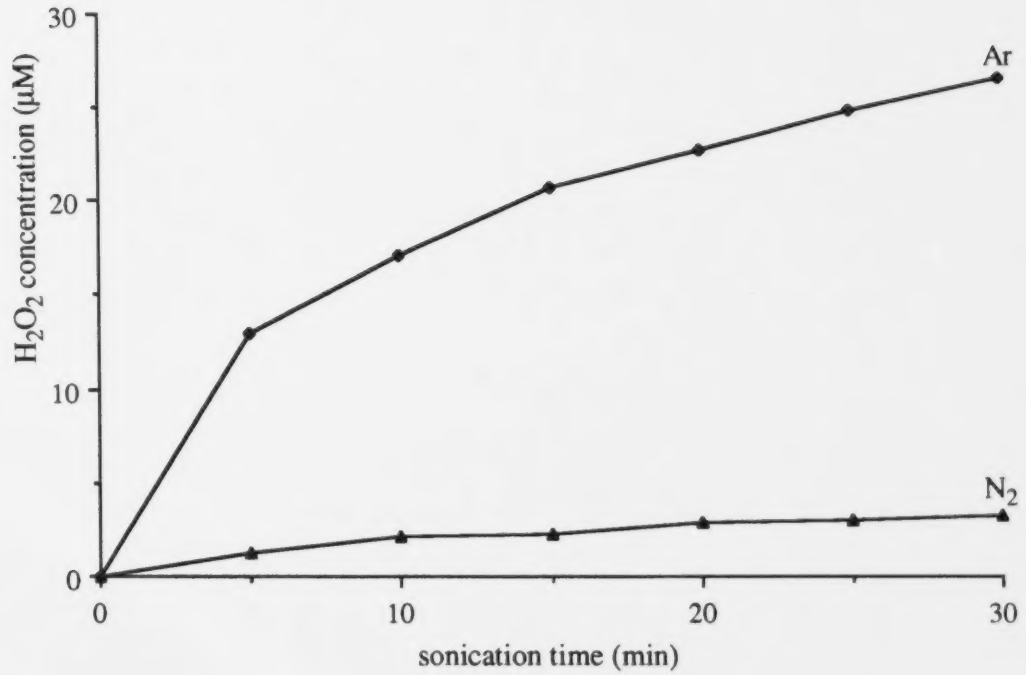


Figure 3.11 Effect of Cavitation Gas Composition on Sonochemical Yields of Oxidants, Reported as Hydrogen Peroxide. n=5; uncertainties at $\pm 1\sigma = 11\%$.

3.3.4.4.1 Measurement of Dissolved Oxygen

A variety of analytical techniques for dissolved oxygen measurements have been proposed in the fields of medicine, biochemistry, soil husbandry, natural water quality control, and in the drug and food industries.[206]

Although the classical Winkler method, first described in 1888,[207] is still in use today, advances in analytical instrumentation have steered most analysts away from the tedious titrations that are involved. Electrochemical methods, especially the Clark membrane covered polarographic oxygen detector,[208] are widely used, as is polarography at a dropping mercury electrode (DME).[209] The great advantage of the DME is that the electrode surface is renewed every few seconds, so that impurity build-up is avoided. However, in complex matrices such as heavily polluted waters the diffusion current may be affected by numerous interfering substances.[210] Due to the simplicity of the matrices encountered in this study, and also to the availability of a ready-to-use DME setup, this method was chosen to monitor oxygen levels prior to and during sonication.

The reduction of O_2 at the DME occurs in two steps. The first wave is due to the reversible reduction of oxygen to peroxide:

$$O_2 + 2H^+ + 2e^- \rightleftharpoons H_2O_2 \quad \text{.....(8)}$$

The second broad and drawn out wave is due to the irreversible reduction of peroxide, formed by reduction of oxygen, to water:

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \quad \text{.....(9)}$$

The first wave was used for these studies.

3.3.4.4.2 The Effect of Oxygen on Formation of Oxidizing Species

Figure 3.12 shows a plot of oxidant formation as a function of sonication time when 80-mL portions of pure water were subjected to various gassing and degassing schemes. A larger vessel was used to accommodate the increased volume, which was twice that used in the previous experiments.

The lower plot shows that solutions degassed by boiling for an hour form negligible quantities of oxidants when subjected to 600 W ultrasound for 25 min. These samples were analyzed polarographically for oxygen content prior to sonication. The results, shown in Table 3.3, indicate that oxygen was not detectable in solutions that were boiled prior to sonication.

When oxygen gas was bubbled through boiled solutions for 5 min prior to and during sonication, the oxidant levels increased (Figure 3.12, middle plot). Polarographic analyses of these solutions, shown in Table 3.3, indicate that an O_2 concentration of 780 μM was present before the commencement of sonication. The presence of oxygen clearly promotes the production of oxidant during sonication, and this observation is consistent with the gaseous cavitation model described above.

The top plot in Figure 3.12 shows oxidant levels in water that was not boiled, but oxygenated for 5 min prior to and during sonication. The results, Table 3.3, indicate that bubbling O_2 had increased dissolved oxygen levels to 940 μM. Levels of oxidants produced by insonation are significantly increased over those seen under previous conditions.

Since cavitation bubbles in solutions in equilibrium with air contain 79% nitrogen (a poor cavitation gas) and only 21% oxygen, it is to be expected that peroxide levels generated during the sonication of samples containing oxygen-equilibrated bubbles would be higher.

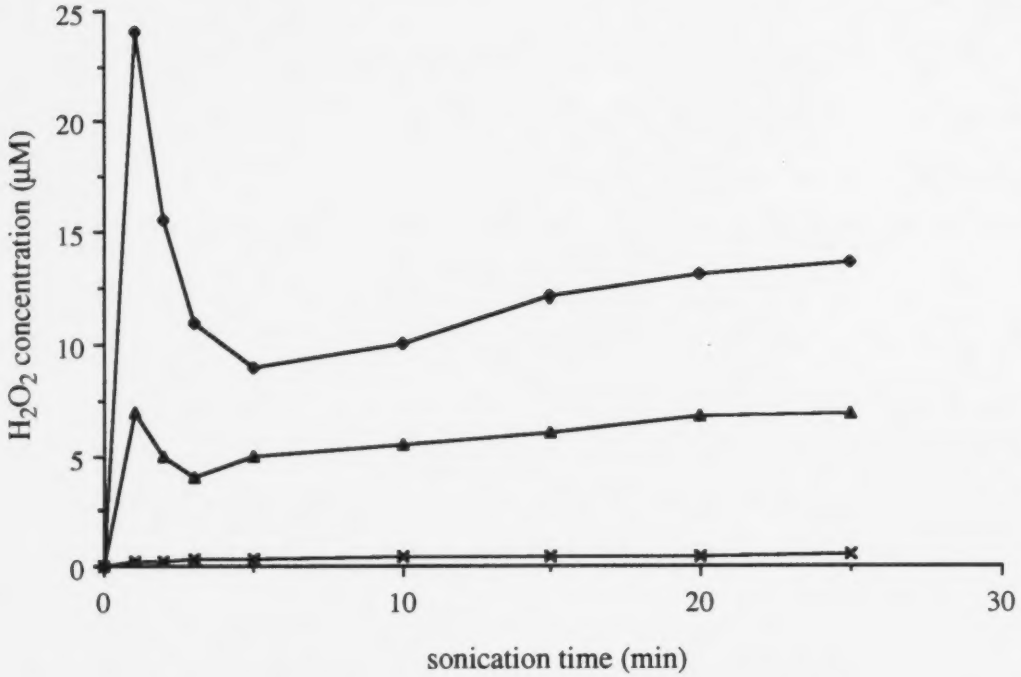


Figure 3.12 Effect of Dissolved Oxygen Concentration on Formation of Oxidizing Species, Measured as Hydrogen Peroxide, in Sonicated Water. ■ = solution in equilibrium with air through which O_2 gas had been bubbled for 5 min prior to, and during, sonication; ◀ = solution boiled to remove dissolved air, then O_2 bubbled through solution for 5 minutes prior to, as well as during, sonication; × = solution boiled to remove all gases, then sonicated in a closed cell. n=3; uncertainties at $\pm 1\sigma$ = 17%.

Table 3.3 Oxygen and Oxidants (as peroxide) Concentration in Unsonicated Water Samples as a Function of Equilibration with Air or O_2 gas.

	Concentration (μM)	
Treatment	O_2 (μM)	H_2O_2 (μM)
Water boiled for 60 min	0	0.00
Water in equilibrium with air	250	0.00
Water boiled for 60 min, then oxygenated for 5 min prior to and during sonication	780	0.08
Water, initially in equilibrium with air, oxygenated for 5 min prior to and during sonication	940	0.23

This is postulated to be due to a "volume effect", i.e., for constant sonication conditions, the number of moles of peroxide produced will be independent of solution volume. As a result, the concentration of peroxide will change with volume. The samples in the oxygen study were all 80 mL, whereas those in the prior experiments were 40 mL. To confirm this point, two sets of nine solutions of water in equilibrium with atmospheric oxygen were taken. No gas was bubbled through any of the solutions either prior to or during sonication. One set was 40-mL, and the other was 80-mL in volume. The solutions were sonicated for varying times, then analyzed for oxidizing power as described before. The resulting profiles, reported as peroxide, are shown in Figure 3.13. The concentration of peroxide for the 80-mL samples was found to be lower than that for the 40-mL samples, although not the 50% predicted. The reason for this discrepancy is not known, and was not studied further. Nonetheless, a comparison of the top plot in Figure 3.12 with the 80-mL plot in Figure 3.13 shows that oxygenation provides higher yields of peroxide.

An interesting feature of the two upper plots in Figure 3.12 is the peak appearing in the first minute or two of sonication. After 5 minutes, the plots take the shape of those observed in samples saturated with air or argon gas. The experiments in Figure 3.13 were repeated twice more and in both trials, done on different days, the results were the same. This suggests that the optimum conditions described in section 3.3.4.2 for the production of oxidants in sonicated water might be more closely approached by the use of oxygen as cavitation gas if relatively large concentrations of peroxide could be maintained.

To determine why the oxidant levels dropped sharply after 3 minutes, the results in air were compared with those for oxygenated samples. When oxygen is present, there is an initial steep rise in oxidant levels over the first 3 to 5 minutes of sonication (Figures 3.7, 3.12 and 3.13).

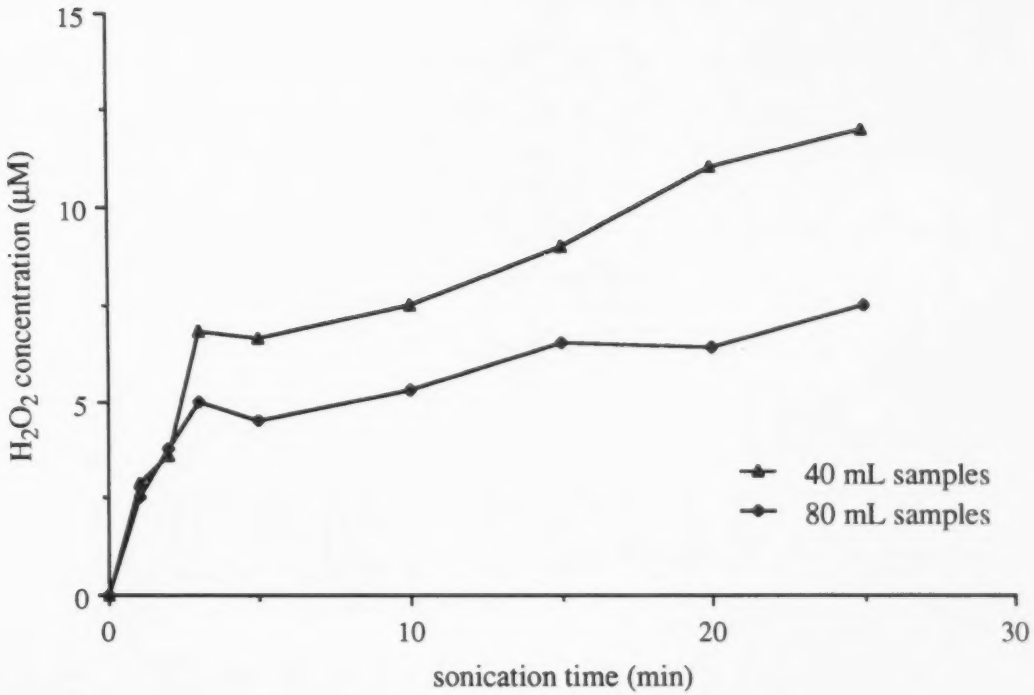


Figure 3.13 Effect of Volume on the Concentration of Oxidants (measured as peroxide) Formed in Water Initially in Equilibrium with Air as a Function of Sonication Time at 600W. n=3; uncertainties at $\pm 1\sigma$ = 13%.

The higher the concentration of oxygen at the beginning of sonication, the higher the initial slope. In air (21% O_2), the slope decreases with time; in oxygen, a peak is observed. This behavior does not occur when cavitation bubbles contain either Ar or N_2 (Fig 3.11).

3.3.4.4.3 Effect of Oxygen Levels During Sonication

Figure 3.14 shows plots of oxygen concentration, measured polarographically, as a function of sonication time for various levels of oxygen exposure. In all cases, dissolved oxygen levels decreased sharply with sonication time during the first 10 minutes of sonication. The effect is more pronounced for samples in equilibrium with atmospheric oxygen prior to sonication. After 25 minutes sonication, the removal of oxygen is essentially complete, except where introduction was continued during sonication. Here the dissolved oxygen levels plateaued at 375 μM. It can be concluded that sonication drives oxygen out of solution rapidly during the first 10 minutes; bubbling pure oxygen through the solution during sonication serves to bring the O_2 concentration to a level equivalent to about 375 μM.

When the peroxide plots in Figure 3.12 are compared with the oxygen plots shown in Figure 3.14, certain trends are noticeable. Oxygen levels are high at the beginning of sonication. Furthermore, the results shown in Table 3.3 show that it is not the O_2 molecule that raises the oxidizing power of sonicated solutions because prior to sonication, the oxygen levels are about 940 μM, but iodimetric analysis of these solutions show less than half a micromolar contribution to the peroxide levels.

The extreme conditions of temperature and pressure achievable during sonication of water have been compared in the literature to those attainable by pulse radiolysis.

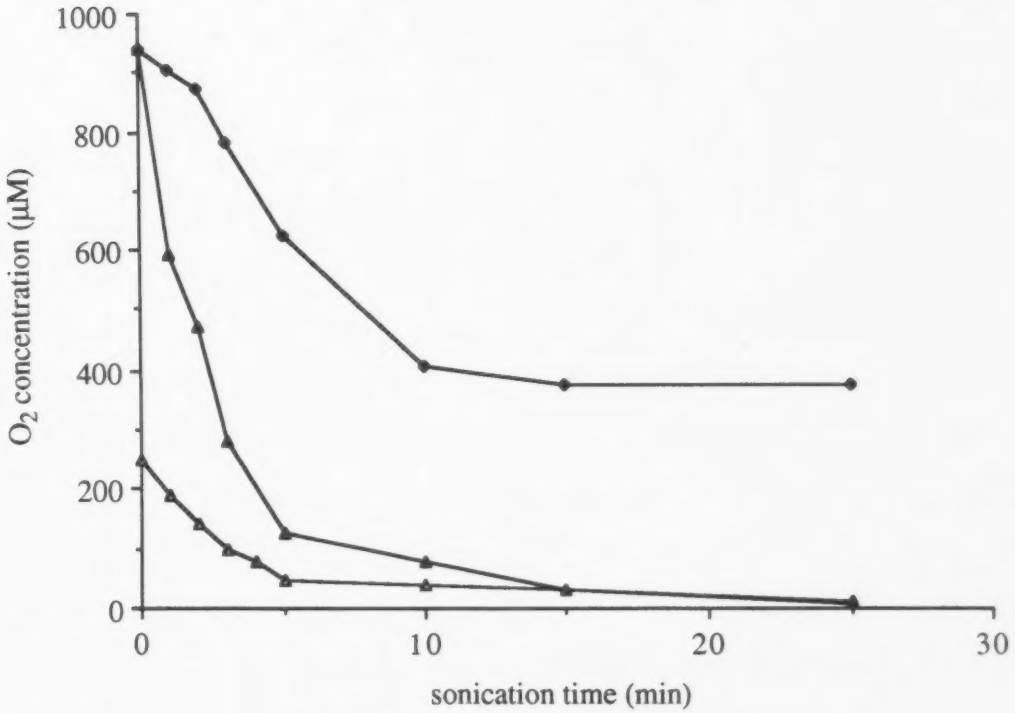
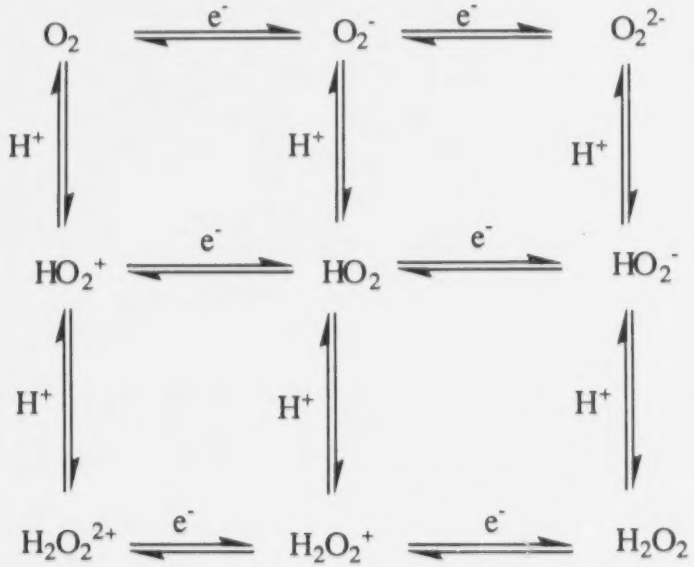


Figure 3.14 Dissolved Oxygen Concentration in Water as a Function of Sonication Time Under Various Levels of Oxygen Exposure. ◆ = solution in equilibrium with atmospheric oxygen that was bubbled with O_2 gas for 5 min prior to, and during sonication; ▲ = solution in equilibrium with atmospheric oxygen that was bubbled with O_2 gas prior to but not during sonication; △ = solution in equilibrium with atmospheric oxygen that was sonicated as is. n= 4; uncertainties at $\pm 1\sigma = 18\%$.

Pulse radiolysis of water produces hydroxyl radicals, hydrogen atoms, and solvated electrons. The existence of free electrons during sonication has been a subject of speculation.[211] If free electrons are indeed formed during cavitation, they may be captured by O_2 to form superoxide anions according to the following scheme:[212,213]



In the presence of protons the O_2^- species forms H_2O_2. This equilibrium scheme could explain why removal of O_2 during the first few minutes of sonication (Figure 3.14) reduces the amount of peroxide formed (Figure 3.12, middle and top plots). It could also explain the pH dependence of peroxide formation during sonication; this is discussed in more detail in section 3.3.7.

In summary, appreciably higher concentrations of oxidizing species are formed in water containing dissolved oxygen than in water that is either degassed or contains nitrogen or argon.

3.3.5 Effect of Temperature on Production of Oxidizing Species

The removal of dissolved gases from solution during sonication arises from two effects. One is through ultrasonic oscillations accelerating diffusion of gas from surrounding liquid into dissolved gas bubbles that are then forced out of solution (sonication is widely used to degas HPLC solvents). Since bubble growth rate and hence degassing is proportional to ultrasound intensity at a given frequency, ultrasound probes can rapidly remove gases from solution. This is disadvantageous to maintaining O_2 levels in solution as well as to producing cavitation. It was shown previously that bubbling a stream of oxygen through a solution during sonication helps to offset the degassing effect somewhat, but does not cause dramatic improvements in the sonochemical yield of oxidants.

Another effect produced when ultrasound energy is passed through water is heating. The mechanical force of ultrasound oscillations causes water molecules to vibrate, thereby converting acoustic energy to heat and producing an overall increase in temperature of the solution. Since the solubility of gases in water decreases with temperature, dissolved gases are driven off until complete degassing occurs at the boiling point of the solvent. Bulk heating due to the passage of high intensity ultrasound through a solution will cause, therefore, dissolved oxygen to be driven off and thereby reduce the number of cavitation sites available. Since the viscosity of the water surrounding the bubble decreases with increased temperature, and the vapor pressure inside the bubble increases, the collapse of any bubbles that remain would likely be less energetic and would be cushioned by the higher vapor pressures inside the bubble.

3.3.5.1 Measurement of Temperature During Sonication

In this part of the study, the temperature of 80-mL portions of water was monitored as a function of sonication time with a mercury thermometer. In one

set of samples, no attempt was made to thermostat the solutions in order to reproduce the conditions under which the results presented so far were obtained. In another set, the sample was immersed in a constant temperature water bath during sonication.

Figure 3.15 shows the results obtained. When the test solutions are not thermostated, the temperature rises rapidly from 23 to 70 °C during the first ten minutes, then more slowly, reaching about 77 °C after 25 min.

Thermostatting produced only a small decrease in the temperature rise, and a value of about 68 °C after 25 min. Clearly the solution is heated rapidly by sonication, and the rate of heat transfer away from the sonicated solution by immersion in a conventional water is not rapid enough to have a significant effect.

When Figure 3.15 is compared to the peroxide and dissolved oxygen plots (Figures 3.12 through 3.14), in all cases the greatest effects occur during the first 10 minutes of sonication. Beyond this time, when little dissolved gas is present in solution, the temperature rise is small. It can be concluded that cavitation, which only occurs when dissolved gases are present, is primarily responsible for these effects.

3.3.5.2 Effect of Temperature on Production of Oxidants by Sonication

The rapid rise in temperature seen in Figure 3.15 is not unexpected. Solutions in ultrasonic baths operating at only 84 W and thermostated at 15 °C have been reported to show an initial sharp thermal rise that plateaus at around 30 °C .[214] The possibility of using temperatures below ambient was rejected because of literature reports[144] that cavitation below 20 °C is very inefficient.

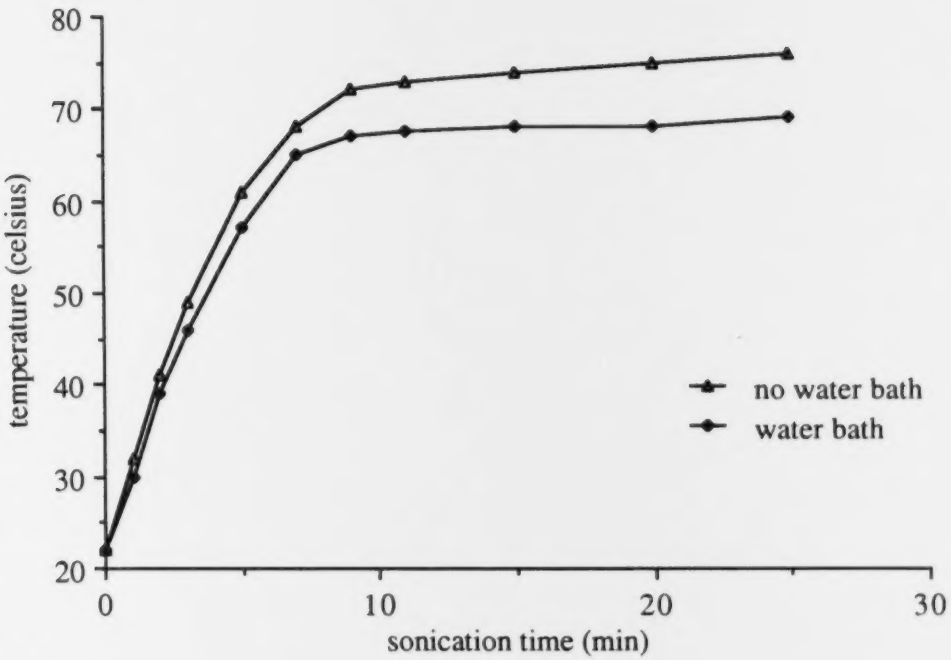


Figure 3.15 Effect of Sonication Time on the Temperature of an 80-mL Sample of Water. Conditions: 600 W power, probe-tip 8 cm from vessel bottom. Reported results are based on single determinations.

As a partial evaluation of the effect of solution temperature on the peroxide plots, the oxidants produced were measured as a function of sonication time for 80-mL water samples immersed in the constant temperature bath. The samples were initially in equilibrium with the atmosphere, and were not oxygenated. The results, shown in Figure 3.16, parallel those seen in Figure 3.13. This result is not surprising since Figure 3.15 shows that the constant temperature bath had little effect on the solution temperature.

3.3.5.3 Effect of Temperature on the Stability of Solutions of Peroxide

Hydrogen peroxide solutions are not stable at high temperature, but undergo decomposition to oxygen and water. It is therefore possible that under the increased temperatures prevalent at long sonication times, decomposition of peroxide may occur. A constant rate of decomposition at a higher temperature could also contribute to the relatively constant levels of peroxide seen at longer sonication times.

To determine the extent to which temperature affects the amount of peroxide produced at long sonication times, several 80-mL portions of a 20 μM stock solution of H_2O_2 were heated on a hot plate. The heating rates were made as close as possible to those shown in the upper curve of Figure 3.15 (non-thermostated). The resulting solutions were cooled, then analyzed spectrophotometrically for H_2O_2.

Table 3.4 shows the results obtained. At 50 °C (temperature observed after 3 min sonication), the concentration was found to have decreased by 14%. Heating the solution rapidly to 75 °C and maintaining it at that temperature for 10 min to simulate the conditions at 25 min sonication resulted in a 50% decrease in the concentration of peroxide. Clearly, aqueous solutions of hydrogen peroxide are not stable to heat.

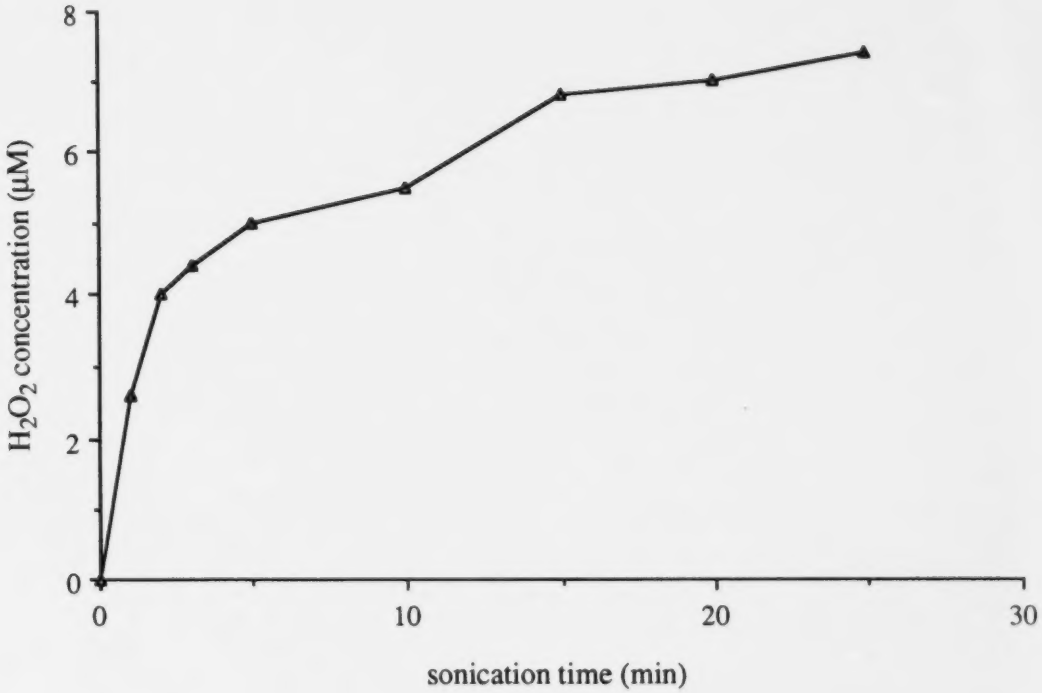


Figure 3.16 Effect of Partial Temperature Control on the Concentration of Oxidants (measured as peroxide) Formed in 80-mL Portions of Water, Initially in Equilibrium with Air, as a Function of Sonication Time at 600 W. Sample vessel was immersed in a constant temperature bath set at 25 °C.

Table 3.4 Effect of Heating on the Concentration of Hydrogen Peroxide Remaining in a Solution Having an Initial Concentration on 0.020 mM.

Temperature (°C)	Concentration found (μM)	Sonication time corresponding to temperature (min)
21	19.85	0
50	17.05	3
75	10.20	25

3.3.5.4 Summary of Temperature Studies

It is clear that dissolved gases play a major role in the production of oxidants in sonicated solutions. In their absence cavitation is minimal, and few if any oxidants are formed. When gases such as argon or nitrogen are present cavitation takes place, but oxidants are produced to only a limited extent. On the other hand, when oxygen is present, significant quantities of oxidizing species are produced. Ultrasound also produces higher temperatures that cause rapid decomposition of peroxide and reduce oxygen solubility. Use of a conventional water bath to thermostat the solutions at around 20°C proved to be ineffective because of the large amount of heat generated during the first few minutes of sonication and the slow rate of cooling through the walls of the sample vessel.

3.3.6 Effect of Acid on H_2O_2 Production

The equilibrium scheme shown in section 3.3.5.4.3 suggests that the production of hydrogen peroxide through gaseous cavitation is likely to have a pH dependence. According to this scheme, the formation of peroxide in the presence of oxygen gas is likely to be more efficient under conditions of high concentrations of protons in the immediate vicinity of the collapsing bubble. The following section describes experiments designed to see whether oxidant levels are improved when sonication is performed in the presence of mineral acids.

3.3.6.1 Sonication in Dilute H_2SO_4

Sulfuric acid is widely used in wet ashing procedures prior to elemental analysis of plant samples. This study aimed to establish whether the presence of H^+ or SO_4^{2-} in the immediate environment of cavitation bubbles affects the production of oxidants.

Varying amounts of concentrated sulfuric acid (17.6 M) were pipetted into water to prepare a set of thirteen solutions ranging in sulfuric acid concentration from 0 to 2.0 M. Sonication was then performed for 25 min on 80-mL portions while oxygen gas was bubbled through the solution. A set of thirteen blanks for the spectrophotometric measurements contained all the reagents without sonication. The oxidant concentrations found were then plotted against sulfuric acid concentration; the results are shown in Figure 3.17. It can be seen from the figure that oxidant formation increases with sulfuric acid concentration up to 0.5 M, then decreases sharply. Relatively little oxidant is generated in 2.0 M H_2SO_4.

Since these observed effects could be caused by either H^+ or SO_4^{2-}, a second run was made replacing H_2SO_4 with Na_2SO_4. The sodium salt was chosen on the basis of its solubility in water and the assumption that sodium ions would not affect sonication significantly. These solutions were sonicated for 25 min and analyzed as before. The results, also shown in Figure 3.17, indicate no observable effect due to either SO_4^{2-} or Na^+ ions.

It can be concluded that the effects observed when H_2SO_4 was added to the samples are due to an increased H^+ concentration. The optimum concentration of H_2SO_4 for oxidant production in this set of measurements was 0.5 M.

3.3.6.2 Sonication in Dilute HNO_3

Nitric acid is also widely used in wet ashing procedures prior to elemental analysis because of its oxidizing strength and solubility of its salts. Therefore its behavior in sonicated solutions is of interest.

A set of solutions ranging in nitric acid concentration from 0 to 2.0 M were prepared and sonicated for 25 minutes as before. The oxidant concentration was then measured, and the results plotted against nitric acid concentration.

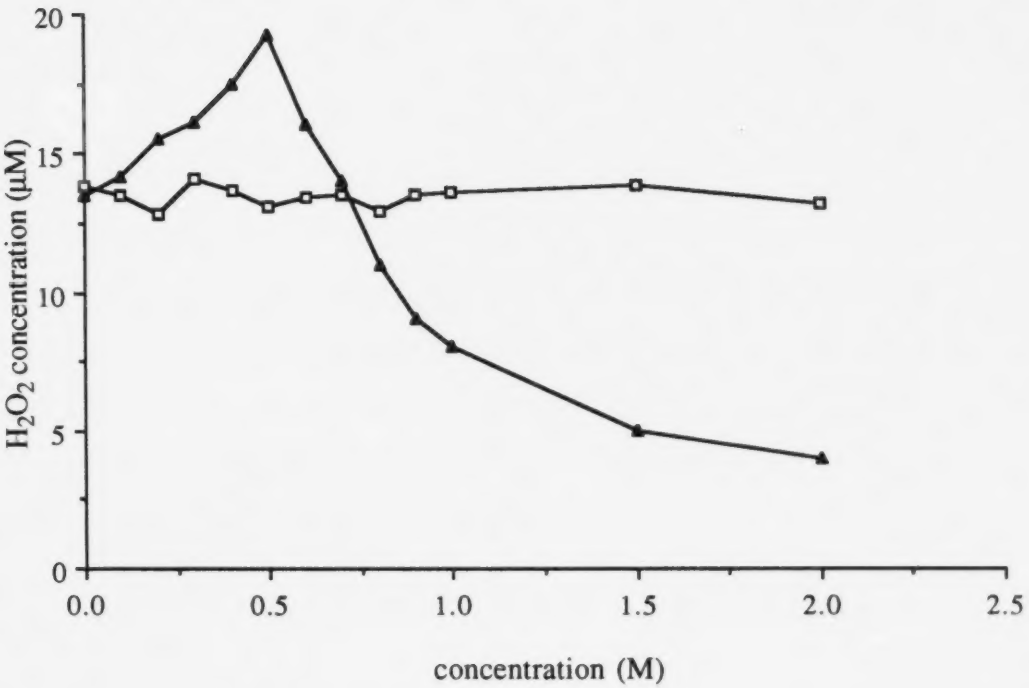


Figure 3.17 Effect of H_2SO_4 (◂) and Na_2SO_4 (▫) Concentration on Formation of Oxidizing Species (measured as hydrogen peroxide). Solutions were sonicated at 600 W for 25 min. n=5; uncertainties at $\pm 1\sigma$ = 12%.

The data, Figure 3.18, reveal a general decrease in oxidant levels with concentration of acid. For the 2.0 M HNO_3 solutions, the odor of nitrogen dioxide could be detected at the end of the sonication period, although the characteristic brown of nitrogen dioxide was not observed.

As with sulfate, the experiment was repeated using $NaNO_3$ in place of HNO_3. The results, also presented in Figure 3.18, show a decrease as the concentration of $NaNO_3$ increases. Little oxidant is found at concentrations of 2.0 M $NaNO_3$. The presence of nitrate ion is clearly detrimental to the production of oxidizing species by the apparatus and conditions used here.

3.3.6.3 Sonication in Dilute HCl

Hydrochloric acid too is widely used in wet ashing procedures, especially as a concentrated combination with nitric acid (a 3:1 ratio of concentrated acids is called aqua regia), prior to elemental analysis because of the complexing ability of the chloride ion with metal ions.

For this study, concentrated hydrochloric acid (12.4 M) or NaCl was added to the samples to prepare sets of solutions ranging from 0 to 2.0 M. The sonication and analytical conditions were as before. The plots, shown in Figure 3.19, indicate a steady decrease in peroxide levels with concentration of acid or its salt.

As with nitrate, increasing concentrations of chloride in the sonication medium lead to lower and lower levels of oxidant production. It may be that nitrate and chloride ions act as radical scavengers[215] to remove hydroxyl radicals immediately after formation in the cavitation bubble, thereby reducing peroxide yields. The nitrate and chloride plots from 0 to 1 M agree with each other within experimental error; above 1 M the line for HNO_3 drops somewhat below the others.

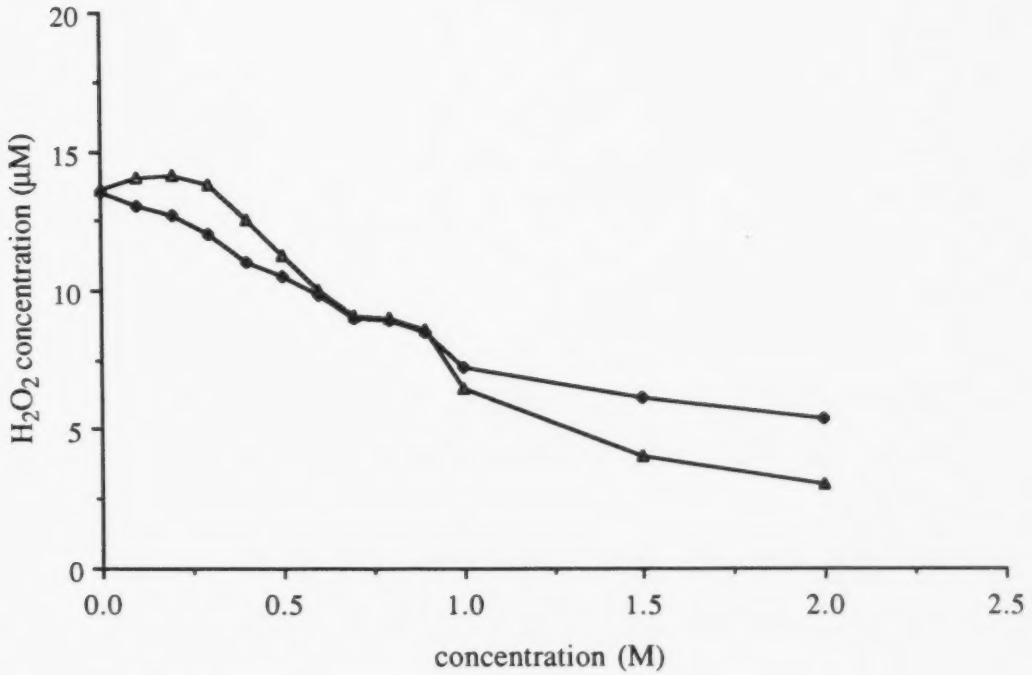


Figure 3.18 Effect of HNO_3 (Δ) and $NaNO_3$ (■) Concentration on Fomation of Oxidizing Species (measured as hydrogen peroxide). Solutions were sonicated at 600 W for 25 min. n=4; uncertainties at $\pm 1\sigma$ = 14%.

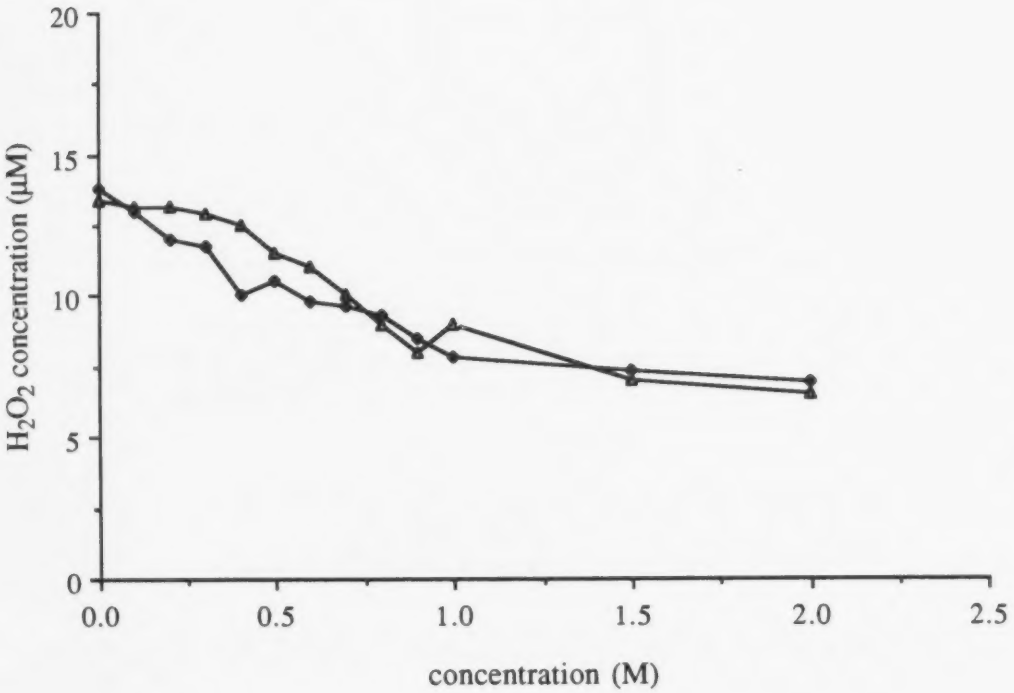


Figure 3.19 Effect of HCl (▲) and NaCl (■) Concentration on Formation of Oxidizing Species (measured as hydrogen peroxide). Solutions were sonicated at 600 W for 25 min. n=4; uncertainties at $\pm 1\sigma$ = 12%.

3.3.6.4 Sonication in Dilute HF

Hydrofluoric acid is often used for the dissolution of siliceous samples to access elements of interest trapped in the silicate matrix. A study was designed to investigate the effect of HF on the formation of oxidants by sonication in the same way as for the other mineral acids. However, sonication of solutions containing even small amounts of concentrated hydrofluoric acid (25.7 M) revealed that the titanium probe was strongly attacked by HF. As soon as sonication was begun, a violent reaction occurred that produced cupious amounts of gas and a dark brown precipitate. After several runs, the surface of the titanium probe in contact with the solution was found to have been eaten away extensively. Therefore experiments with HF were discontinued, and work on NaF was not undertaken.

3.3.6.5 Sonication in Mixtures of Acids

In a majority of wet ashing procedures that incorporate acids for digestion of solid samples, a combination of more than one acid, depending on the application, is employed. For example, the total dissolution of soils may require a combination of nitric, perchloric and hydrofluoric acids. In this example nitric acid is added to decompose easily oxidisable organic material, perchloric acid, when hot and concentrated, to destroy the remaining oxidation-resistant organics, and hydrofluoric acid to decompose the siliceous material.

The aim of this study is to determine the effect of acid mixtures on the generation of oxidising species by sonication. A mixture of special interest was HNO_3 and HF because of the utility of these acids in dissolution. However, preliminary experiments showed that these mixtures were not applicable to sonication for the following reasons:

(i) For low concentration ranges of HNO_3 (from 0 to 0.5 M), increasing the amount of HF in the reaction mixture increases the extent to which the Ti probe is eroded and an insoluble brown residue formed.

(ii) At high concentrations (>1.0 M) of both HNO_3 and HF, large volumes of brown N_2O_4 are produced as soon as sonication is initiated.

Formation of nitrogen oxides is undesirable for two reasons. Firstly, they are highly corrosive to the electrical and mechanical components of the ultrasonic probe. Secondly, through their initial formation and removal from the solution, the initial concentration of HNO_3 is decreased, and thereby not available for oxidation of the sample.

Sonication of HF mixtures has the added factor of a safety hazard owing to the violence of the reaction. It was found necessary during some runs to either lower the probe power considerably, or periodically quench the reaction with a stream of water when it became too violent during sonication. Close attention was required at all times.

3.4 CONCLUSIONS

The results obtained in this study indicate that highly reactive intermediates, probably including hydroxyl radical and hydrogen peroxide, are produced when ultrasound energy is propagated into water at high power. Cavitation is the primary process by which these intermediates are formed; for it to occur, dissolved gases must be present to provide nucleation sites.

The effect of dissolved O_2, N_2, and Ar on oxidant formation was investigated. Argon was found to provide the most energetic internal bubble environment during the compression phase of the wave, whereas nitrogen was found to

provide the least energetic environment, at sonication times greater than 10 minutes. On the other hand, oxygen was found to produce the highest levels of oxidising species during the first 3 minutes of sonication, but at longer sonication times, elevated temperatures and degassing led to reduced peroxide yields. In all cases studied, replacement of gas lost through ultrasound degassing by bubbling through an inlet tube was found to improve oxidant yields. Attempts to cool the solutions in a constant temperature bath during sonication proved inadequate because at the power levels delivered by the probe, solution heating rates were too high to be offset by a conventional thermostated jacket around the sonication cell.

Finally, the addition of acid to the water being sonicated improves the oxidant yield. An acid concentration of 1.0 M was found optimum. The anion associated with the protons was also varied. The presence of SO_4^{2-} was found to have no effect on the oxidant yield. The presence of free radical scavengers such as chloride reduce yields, as did nitrate. HF attacked the titanium probe and generated a violent reaction that posed safety hazards.

Perhaps the most significant conclusion that can be made from this study is that in addition to inducing mechanical erosion of solid substances suspended in a sonication solution, ultrasound also induces chemical changes in water that make it more reactive towards substances dissolved in it. This has useful implications for the dissolution of samples, especially organic materials, since hydroxyl radicals and hydrogen peroxide are good oxidants for many organic compounds. The results presented here suggest that under selected conditions of ultrasonic power, temperature, cavitation gas composition, and probe positioning, ultrasound may decompose biological solids with little or no addition of external reagents. The potential advantages of an approach using *in-situ* generation over traditional approaches to dissolution include elimination or reduction in the use

of acids and of the introduction of impurities from the reagents. Also, dissolution rates may be speeded up. This idea is explored in the following chapter.

CHAPTER IV

STUDIES OF EFFECT OF SOLVENT COMPOSITION ON DISSOLUTION OF BIOLOGICAL MATERIALS: ULTRASOUND VERSUS HEAT

4.1 INTRODUCTION

The results presented in Chapter II indicated that sonomechanical phenomena arising from cavitation may aid in the dissolution of solid samples; the results discussed in Chapter III indicated that, with careful control of the internal and external environments of cavitating bubbles, oxidants that may aid in the dissolution process can be produced in significant quantities. In this chapter, advantage is taken of these findings to develop an ultrasound-based method for dissolution of solid biological materials.

4.1.1 Biological Sample Dissolution: Ultrasound vs Heat Energy

The use of ultrasound provides a unique and potentially powerful new mechanism for sample dissolution. Ultrasound-induced cavitation produces shock-waves and microjets of solvent directed towards solid particles suspended in a liquid which erode particle surfaces and break up larger particles. This physical process is important because it increases the solvent-accessible surface area of the sample and may enhance rates of dissolution. Cavitation in water as solvent also produces oxidising species, possibly hydroxyl radicals and hydrogen peroxide. This chemical process is also important in that enhanced

dissolution rates, especially of organic matrices such as biological materials, may be possible without external reagent addition, or with smaller quantities.

The use of heat as a source of energy for dissolution of samples does not have the advantages outlined above. Mechanical break up of sample particles is less likely to occur during the dissolution process, and as a result, a separate grinding step *before* dissolution is often used as a means of achieving larger surface areas to accelerate digestion rates. Also, at the temperatures normally used for hot-plate digestions, few chemical changes that can aid in dissolution occur to the solvent. The application of heat acts primarily to increase the number of collisions between particles of sample and externally added reagents, and also to provide sufficient energy to overcome activation barriers between sample and reagent molecules.

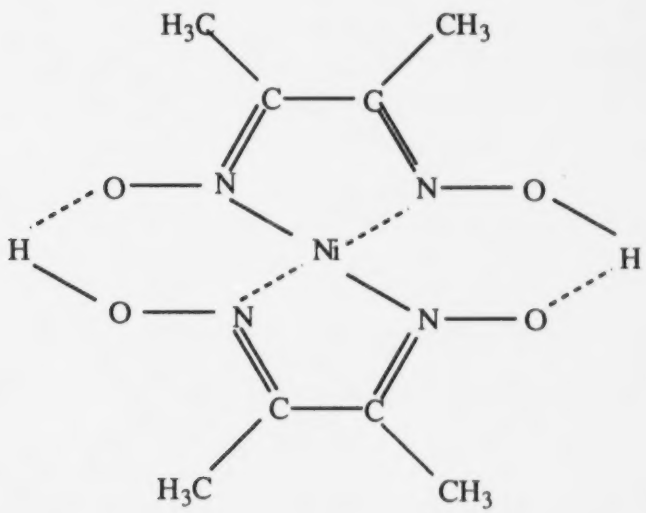
On the basis of these facts, dissolution rates may be higher when ultrasound energy is used in place of heating on a hot plate. This chapter describes studies comparing the two approaches. Since hydroxyl radicals and hydrogen peroxide rapidly decompose many organic compounds, the studies were directed toward dissolution of biological materials in water as solvent.

4.1.2 Method Development

4.1.2.1 Use of Model Compounds and Certified Reference Materials

Many metals found in biological fluids and tissues exist in the form of complexes with biological ligands. Some of these complexes, such as the metal porphyrin chelates (chlorophylls, vitamin B_{12}, hemoglobin, etc.) are highly stable. To determine total metal concentrations in biological tissues by atomic spectroscopic methods such as FAAS and solution-based ICP-OES, the metal-ligand bond of these complexes may have to be broken in order to release the metal into solution prior to nebulization.

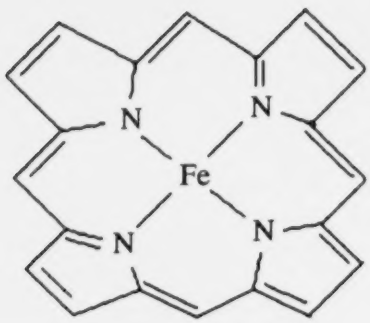
Two chelates, bis(dimethylglyoximato nickel)(II) ($Ni(DMG)_2$) and hemin, were selected as model compounds representing stable complexes that are likely to be encountered in biological materials. $Ni(DMG)_2$ is a strawberry red chelate in which the central nickel atom is coordinated to four nitrogen atoms in a square planar arrangement (structure I).



Structure I

This complex was chosen for study because it is highly insoluble and stable in water, and is well characterized and inert.[216]

Hemin is a high molecular weight porphyrin complex in which iron is coordinated to a tetrapyrrole nucleus (structure II).



Structure II

Hemin is the biologically active portion of the hemoglobin molecule. It is synthesized in the liver and concentrated in red blood cells for oxygen transportation. Hemin is obtained from bovine liver or blood as insoluble dark blue crystals. Like all biological complexes in its class, hemin has a high thermodynamic stability and the rate of exchange of iron is very slow. According to Falk and Phillips,[217] nothing less than concentrated H_2SO_4, to which the porphyrin nucleus is stable, will remove iron from the tetrapyrrole ring. Compared to chlorophyll and vitamin B_{12}, hemin is relatively inexpensive and obtainable in high purity, an important consideration when doing evaluations requiring repetitive analyses.

After method development using the model compounds described above, the approach suggested by Sutarne and Steger[218, 219] was used to evaluate the performance of the proposed technique. These authors recommend the use of certified reference materials to assess the reliability and validity of proposed analytical methods for the determination of major, minor and trace elements in a variety of materials. For this work, the reference materials TORT-1, Lobster hepatopancreas, and SRM #1568a, Rice Flour, were used.

Since a large number of analyses, each involving several elements, was anticipated, a multielemental measurement system was considered desirable. Therefore inductively coupled plasma atomic emission spectrometry was selected as the analytical method. Background information about ICP-OES is given below.

4.1.2.2 Multi-element Determinations Using the ICP as an Excitation Source

The analytical method generally employed for the determination of metals in biological tissues is emission spectroscopy.[33] Many analytical procedures have been developed using an inductively coupled plasma (ICP) as an excitation source for multi-element spectroscopic measurements.

An ICP produces high temperature through the effect of a radio-frequency field on a flowing gas. Typically, a copper coil is mounted around a borosilicate torch, and 1 to 2 kW of 27 MHz radio frequency power is applied while a gas (typically argon) is allowed to flow concentrically around the central tube of the torch. The resulting temperature of around 10,000 K is sufficient to transform a sample from its initial state as solid, liquid, or gas into a plasma of atoms, ions, and molecular radicals that are electronically excited. De-excitation of these excited states produces emission spectra that can be used for both qualitative and quantitative analysis of the sample. Samples may be injected into the gas pathway as solids or as liquids, but the most popular approach is to aspirate liquid samples after nebulization. This approach allows the use of conventional pneumatic nebulizers and spray chambers from atomic absorption instruments to produce a fine mist of sample droplets. These droplets are dried and atomized as they are carried into the ICP torch by a stream of carrier gas.

In the 10,000 K atmosphere of the ICP discharge, all compounds present in the sample are likely to be completely atomized, so chemical interferences are

negligible. However, spectral interferences from the discharge are possible; these often can be reduced by optimizing the height of the detector photon receiving area above the load coil. Linearity of the analytical curves exceeds that obtainable in atomic absorption methods by several orders of magnitude, enabling the technique to simultaneously measure elements in ppb as well as % levels without further dilution of the sample. Detection limits for most elements measurable by ICP are much lower than those obtained by FAAS. Except for the halogens, which require very high excitation temperatures, almost all of the elements in the periodic table can be determined simultaneously using a direct reader ICP spectrometer.

For these reasons, all elemental determinations were conducted using the ICP. Details of the operating parameters are described in section 4.2.4 below.

4.2 EXPERIMENTAL

4.2.1 Materials

Two times crystallized, dialyzed and lyophilized bovine hemin was supplied by Sigma Chemical Co. (St. Louis, Mo, USA). The marine biological reference material TORT-1, prepared from acetone-extracted lobster hepatopancreas, was obtained from the Marine Analytical Standards Program, Division of Chemistry, National Research Council (NRC), Ottawa, Canada. Standard Reference Material #1568a, Rice Flour, was supplied by the National Institute of Standards and Technology (formerly the National Bureau of Standards, Gaithersburg, MD, USA).

To prepare $Ni(DMG)_2$, 20 mL of a 100 ppm solution of nickel (in HNO_3 matrix), was neutralized by addition, with stirring, of enough 2.5 M NaOH to

make the solution slightly basic, followed by addition of 10 mL of pH 6.5 acetate buffer solution and 20 mL of 1% solution of dimethylglyoxime in ethanol. The precipitate was then washed several times with 10-mL portions of water. No attempt was made to dry the precipitate prior to use.

4.2.2 Solutions

Ultrapure water (18.3 MΩ.cm) from a Barnstead D4751 NANOpure@ ultrafiltration system was used throughout the study. Working metal standards for ICP-OES were prepared by dilution of 1000 μg/mL stock solutions of Cd, Sr, Cu, Fe, Mn, Ca and Mg (Fisher Scientific Co.). All other reagents - CCl_4, HCl, HNO_3, HF, H_2SO_4 and H_2O_2 - were analytical grade (BDH Chemicals, Toronto, Canada).

All glassware was soaked overnight in 3% HNO_3 and rinsed with NANOpure@ water prior to use.

4.2.3. Sonication Procedure

The sonochemical reactor assembly shown in Figure 3.1 and described in section 3.2.2.1 was used. The only modification was the use of a 0.5" diameter commercial probe. In order to have as much sample directly under the probe as possible, decompositions were carried out in flat-bottomed narrow cylindrical vessels (11 cm long, 3 cm diameter, 80-mL capacity) with a probe to vessel bottom distance of 8 cm.

4.2.4. ICP Atomic Emission Measurements

Sonicated solutions, blanks and standards were aspirated into a direct-reader ICP-OES instrument (ARL 34000 Applied Research, N.J., USA). The ICP source is a 2.5 kW RF generator operating at 27 MHz. The spectrometer optical system

is based upon a 1-meter Rowland circle with a 1080 lines/mm holographic grating on a Paschen Runge mount. Data acquisition is through a 5 digit charge transfer ADC at a nominal rate of 50 channels per second. Data were acquired by a dedicated PDP-11/03 minicomputer (Digital Equipment Corporation) and processed by an IBM-AT microcomputer. A standard pneumatic nebulizer was used to aspirate all blanks, samples and standards. To avoid sample carry-over, the spectrometer was programmed to sample the signals after a 10 sec aspiration time, and to integrate the signals for the same duration. Emission signal intensities were recorded in mV units, ranging from 1 to 15000 mV, with an accuracy of 1 mV. Standards were run before and after each set of samples. The ratio of signal intensities between samples (after blank correction) and standards was used to determine the concentration of elements in the sample.

It was shown in the last chapter that significant yields of oxidizing species can be obtained in sonicated aqueous solutions by careful optimization of the factors that affect cavitation. These include making the solvent system 0.5 M in H_2SO_4, and continuously bubbling a small stream of oxygen gas through the suspension during sonication. The goal of this study is to demonstrate this capability on $Ni(DMG)_2$ and hemin, and to show that the method can be successfully applied to biological reference materials.

4.3 RESULTS AND DISCUSSION

4.3.1. Study of the Effect of Ultrasound on the Dissolution of $Ni(DMG)_2$

4.3.1.1 Calibration of the ICP-OES for Ni

A set of 0, 10 and 100 ppm solutions of nickel were aspirated into the ICP and the detector readings recorded. A blank correction was made by subtracting the

absolute signal for water from the absolute signals for the standards. The net signal intensities were: for 10 ppm, 5.9 mV, and for 100 ppm, 63.5 mV. The calibration plot, shown in Figure 4.1, indicates that there is good linearity within this concentration range; the dynamic range of the ICP for this element covers at least 2 orders of magnitude.

4.3.1.2 Study of Possible Ni Contamination from Ultrasonic Probe

Prior to sonication of $Ni(DMG)_2$ suspensions, nickel contamination by the probe was investigated. A new 0.5" diameter titanium tip was installed and the probe immersed in approximately 60 mL of 0.5 M H_2SO_4. Sonication was carried out at a probe-vessel bottom distance of 4 cm and at full power for 5 min to remove impurities that might be absorbed on the surface of the tip. After rinsing with NANOpure@ water, a fresh 50-mL portion of 0.5 M H_2SO_4 solution was sonicated at the same power level but at a solution depth of 8 cm for 120 min. The resulting solution was quantitatively transferred to a 100-mL volumetric flask and diluted to volume prior to aspiration. To determine the amount of nickel introduced by the probe in the absence of acid, a 50-mL portion of NANOpure@ water was sonicated under the same conditions for 120 min. The ICP-OES signal intensities for nickel in these solutions are compared to that of an unsonicated water sample in Table 4.1. These results indicate that neither sulfuric acid nor the probe, introduced nickel contamination during 120 min of sonication.

4.3.1.3 Sonication of $Ni(DMG)_2$ in $H_2O/CHCl_3$ Mixtures

Solid $Ni(DMG)_2$, prepared as described in section 4.2.1, was dissolved in 20 mL of chloroform, followed by addition of 50-mL NANOpure@ water. The resulting $H_2O/CHCl_3$ mixture was sonicated at full power with a tip to cell bottom distance of 8 cm while oxygen was passed through the solution.

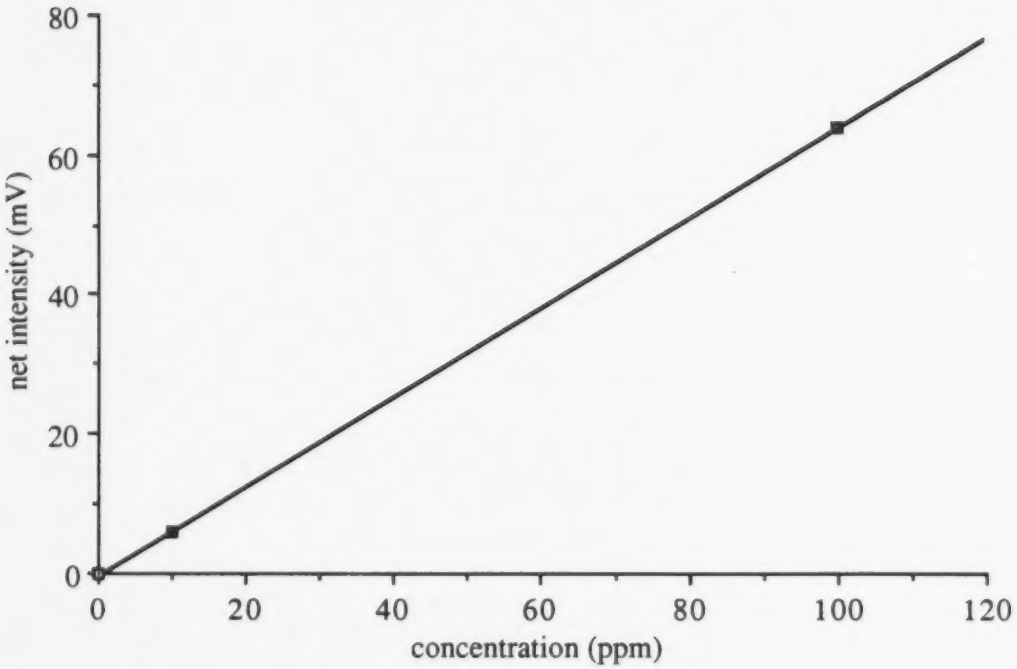


Figure 4.1 ICP calibration Curve for Nickel.

Table 4.1 Effect of Sonication and Addition of Acid on ICP-OES Signal Intensity for Nickel.

solution	sonication time (min)	absolute intensity (mV)
H_2O	0	21.6
H_2O	120	21.4
0.5 M H_2SO_4	120	21.7

The aim of introducing the chloroform phase was to keep the $Ni(DMG)_2$ in this phase while retaining all free Ni in the aqueous phase. The extent to which ultrasound had decomposed the complex could then be determined by ICP-OES measurements of the aqueous phase.

The effect of ultrasound on the solvent mixture was to very rapidly convert the two phases into a cloudy white emulsion. This observation is consistent with literature reports of similar experiments on palm oil/water mixtures.[178] Also, very rapid evaporation of the chloroform layer occurred, so that only about 5 mL of the $CHCl_3$ phase remained after 30 min of sonication.

The aqueous phase was then carefully separated from the organic phase and transferred to a 100-mL volumetric flask. After dilution to volume, the solution was aspirated into the ICP. Table 4.2 shows the ICP-OES results for Ni in the aqueous phase at 0, 5, and 30 min of sonication. It can be seen that the Ni concentration in the aqueous phase increases with increasing sonication time; approximately 400 μg of Ni are released into the aqueous phase after 30 min of sonication.

It is not known whether the nickel signal observed is due to free Ni released into the aqueous layer by ultrasound decomposition of the $Ni(DMG)_2$, or to $Ni(DMG)_2$ present in the water phase, either as a solute or in dispersed microdroplets of $CHCl_3$. The important point to note is that the amount of nickel in the water phase is small but increasing with sonication time.

4.3.1.4 Sonication of $Ni(DMG)_2$ in 0.5 M H_2SO_4

This investigation was conducted with the aim of assessing the effect of dilute sulfuric acid on the release of nickel from $Ni(DMG)_2$. It was established in Chapter III that the formation of hydrogen peroxide during sonication of water was enhanced when the system was made 0.5 M in H_2SO_4.

Table 4.2 Total Amount of Ni Found in Aqueous Phase After Sonication of $Ni(DMG)_2$ in $CHCl_3/H_2O$ Mixtures.

sonication time (min)	amount of Ni released into H_2O phase (μg)
0	0
5	145
30	377

It was therefore expected that a larger Ni signal would be observed for a sonicated suspension of $Ni(DMG)_2$ in 0.5 M H_2SO_4 than for the same suspension in water.

Solid $Ni(DMG)_2$ was prepared as described in section 4.2.1. In this study, the precipitate was suspended in 50 mL of 0.5 M H_2SO_4. The acidic mixture was then insonated at full power while oxygen was passed though the solution. A clear colorless solution with no traces of solid was produced in less than 3 minutes.

To determine whether this was due to decomposition of the ligand through oxidation by ultrasound-produced peroxide and free radicals, or through protonation of the ligand to release the central Ni atom, the acidic suspension of $Ni(DMG)_2$ was mechanically shaken by hand instead of being sonicated. The precipitate was found to slowly dissolve over a period of about 2 hrs to give a clear colorless solution. We conclude that the complex is unstable in dilute sulfuric acid. When 5 mL of 2.5 M NaOH was added to both the sonicated and mechanically shaken solutions to neutralize the acid, $Ni(DMG)_2$ reprecipitated; this indicates that the release of nickel is caused by protonation of the ligand rather than by decomposition of the ligand. The acid may be protonating the nitrogens in the ligand and releasing free Ni into solution, regardless of whether the mixture is being sonicated or mechanically agitated.

4.3.1.5 Sonication of $Ni(DMG)_2$ in Water and 0.5% H_2O_2

To determine whether oxidants produced during sonication do contribute to the break up of $Ni(DMG)_2$, two sets of experiments were performed. In one set, the complex, prepared using the procedure described in section 4.2.1, was suspended in 100 mL of water and 2 mL of 30% H_2O_2 added to make the solution approximately 0.5% in H_2O_2. Half of the resulting suspension was

heated at 100°C on a hot plate for 30 min, while the remaining portion was sonicated for the same time interval. In the other set, the procedure was repeated, but without hydrogen peroxide.

The results of nickel determinations in these solutions are presented in Figure 4.2. In both cases, sonication produced significantly higher amounts of free nickel than heating. The complex is stable and insoluble in water, but is unstable in 0.5% H_2O_2. Furthermore, the results obtained for ultrasound treatment of the complex in water are only slightly less than those obtained when the complex is heated in 0.5% peroxide. This result corroborates the observation in Chapter III that ultrasound in water produces oxidants in significant quantities. Reports of oxidative degradation of organic compounds such as pentachlorophenol[220] in sonicated water samples have recently been published; in a majority of the cases, ultrasound-produced hydroxyl radicals were held responsible for the enhanced degradation rates.

In the experiments described in this section, addition of NaOH to make the solutions basic after sonication or heating did not cause reprecipitation of the $Ni(DMG)_2$. The release of Ni in this case is likely the result of chemical decomposition of the organic portion of the complex.

The mechanism of decomposition in these solutions was not studied further. It may be that ultrasound is splitting peroxide to form highly reactive hydroxyl radicals, or that the number and effectiveness of collisions between reactants produced by ultrasound is magnified by the physical action of ultrasound, or even that the reaction pathway in the presence of ultrasound is different than in its absence. The possibility that all of these factors are working in combination to produce the observed ultrasonic effect can not be ruled out either.

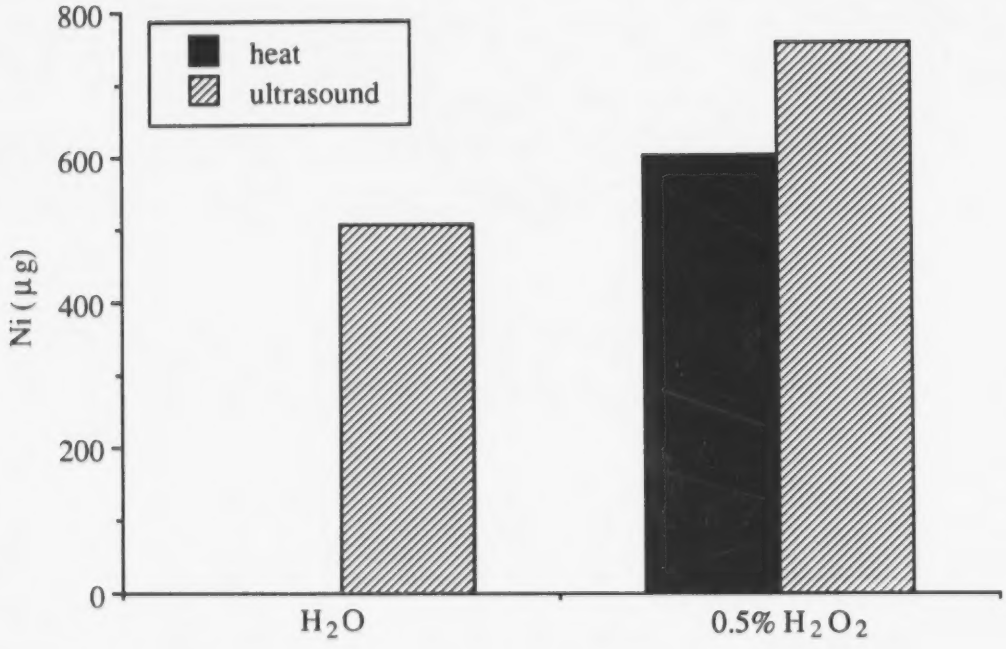


Figure 4.2 Quantity of Free Nickel Released Upon Sonication or Boiling for 30 minutes of 50 mL of Solution Containing 2 mg of Nickel Initially as $Ni(DMG)_2$. No measurable free nickel was found in the heated aqueous sample.

4.3.1.6. Summary of $Ni(DMG)_2$ Results

The results above clearly show that the release of nickel from $Ni(DMG)_2$ in water when sonicated is in part due to decomposition of the organic portion of the complex by ultrasound-produced oxidants such as hydroxyl radicals and/or hydrogen peroxide. Dilute sulfuric acid aids in the release of nickel by protonating the nitrogens of the ligand. Because the complex studied here is unstable under the optimum conditions of cavitation described in Chapter III, (0.5 M H_2SO_4), it does not provide clear cut conclusions as to whether ultrasound-produced free radicals and hydrogen peroxide will achieve decomposition and bond-cleavage to release the free metal. The following section reports on a study of hemin, a compound that does not dissolve in dilute sulfuric acid even after heating at 100 °C for several hours.

4.3.2 Comparison of Ultrasound and Heat for the Decomposition of Hemin

A series of experiments were conducted to establish conditions for the rapid and quantitative ultrasonic dissolution of bovine hemin. As stated earlier, hemin is insoluble and apparently unaffected by dilute sulfuric acid solutions at room temperature. It is therefore a more suitable model compound for this study.

4.3.2.1 ICP-OES Calibration for Fe

Figure 4.3 shows a calibration curve obtained when 0, 10.1 and 101 ppm solutions of iron were aspirated into the ICP. The signal intensities, after blank subtraction as in section 4.3.1.1, were: for 10.1 ppm, 450.25 mV, and for 101 ppm, 4281.95 mV. There is good linearity within this concentration range; the dynamic range of the ICP for this element covers at least 2 orders of magnitude. The large slope also indicates excellent sensitivity for Fe determinations.

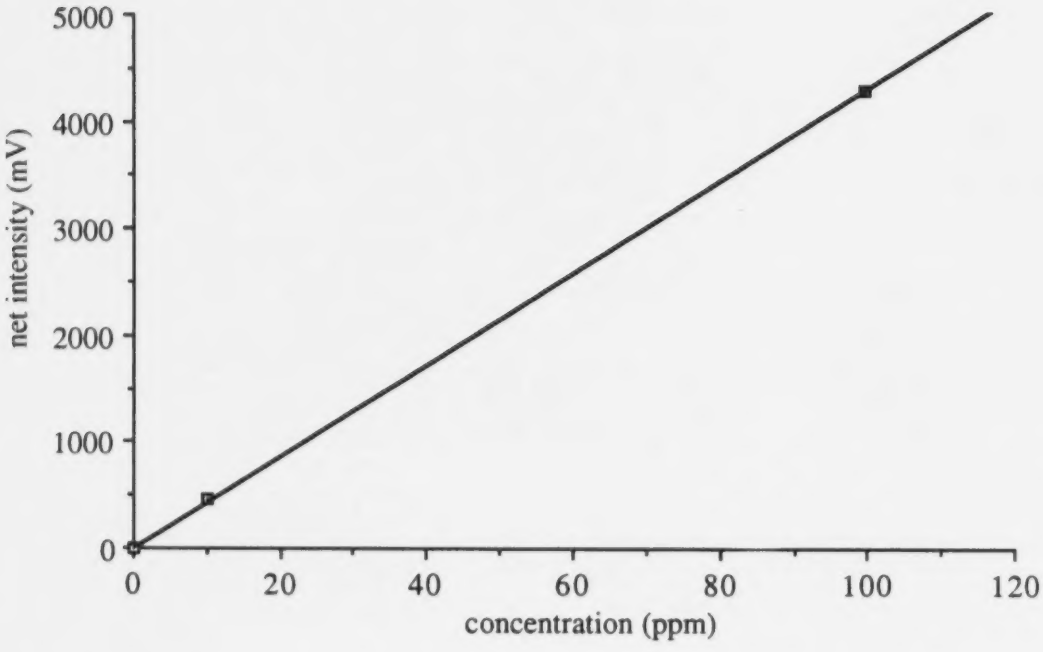


Figure 4.3 ICP Calibration Curve for Fe.

4.3.2.2 Effect of Concentrated Acid on Hemin

The behavior of hemin in concentrated solutions of several mineral acids was investigated. To 100-mg portions of hemin were added 10 mL of concentrated HNO_3 (15.4 M), H_2SO_4 (17.6 M) or HCl (12.4 M). The mixtures were then heated on a hot plate at 100 °C for 30 min. Samples were then diluted to 100 mL, filtrered and analyzed for Fe by ICP. A 10.1 ppm Fe standard was run before and after the samples. The ratio of the Fe emission signal from the sample to that from the standard was used to determine the amount of Fe in solution. To calculate the % dissolution, this value was compared to that expected if all the hemin iron were released.

In HCl, hemin crystals remained unchanged during 30 min of heating, and the filtrate was clear. The results shown in Table 4.3 indicate that an insignificant amount of iron was released into solution. Hemin is therefore stable in concentrated HCl.

In nitric acid however, dissolution, accompanied by the release of a brown gas, occurred rapidly, and was essentially complete in 30 min. No solid residue remained, even though the resulting solutions were slightly green in color. The results, also shown in Table 4.3, show that almost all the hemin iron was released. This result is not surprising, since nitric acid is a strong oxidizing agent when concentrated.

In concentrated sulfuric acid, dissolution of hemin also occurred, but to a lesser extent (see Table 4.3). This result is also not surprising, since concentrated sulfuric acid is a powerful dehydrating agent. A dark colored solid residue was formed during heating (probably carbon particles formed by charring of hemin), and the color of the supernatant was light brown.

Table 4.3 Release of Iron From Hemin in Concentrated Mineral Acids. Mixtures were heated for 30 min at 100 °C.

acid	% dissolution
HCl	0.01
HNO_3	98
H_2SO_4	85

4.3.2.3 Effect of Heat and Ultrasound on Hemin in Water

Two sets of experiments were performed to determine the effect of heat and ultrasound on hemin in water. In the first set, 100-mg portions of hemin were placed in sonication vessels, 50 mL of NANOpure@ water added to each, and ultrasound passed through each suspension for specific times. In the second set, the same quantities of hemin and water were placed in a series of beakers and heated at 100 °C, again for various times. The Fe released into each solution was then measured by ICP after passage through a 4-8 μm sintered glass filter to remove particulates. The results, plotted in Figure 4.4, show that only a small amount of free Fe (3.6 μg, about 0.004% of the total) is observed after heating for 3 hrs. On the other hand, sonication for the same period of time leads to release of about 0.5% of the expected amount of Fe. This result is not unexpected because oxidants produced by ultrasound energy when propagated into water (Chapter III) are likely to decompose some of the hemin.[220] Also, the increase in surface area of hemin that is accessible to the oxidising species assists the process.

4.3.2.4 Effect of Heat and Ultrasound on Hemin in Dilute Acid

The effect of acid on the formation of oxidants in sonicated water was investigated in Chapter III; solutions of 0.5 M H_2SO_4 were found to produce better yields of peroxide than HCl and HNO_3. In this study, the effect of dilute acid on the decomposition of hemin when subjected to ultrasound and heat is investigated.

The procedure described in section 4.3.2.3 above was repeated on 100-mg portions of hemin in dilute solutions of HCl, HNO_3 and H_2SO_4. The results are shown in Figures 4.5 through 4.7.

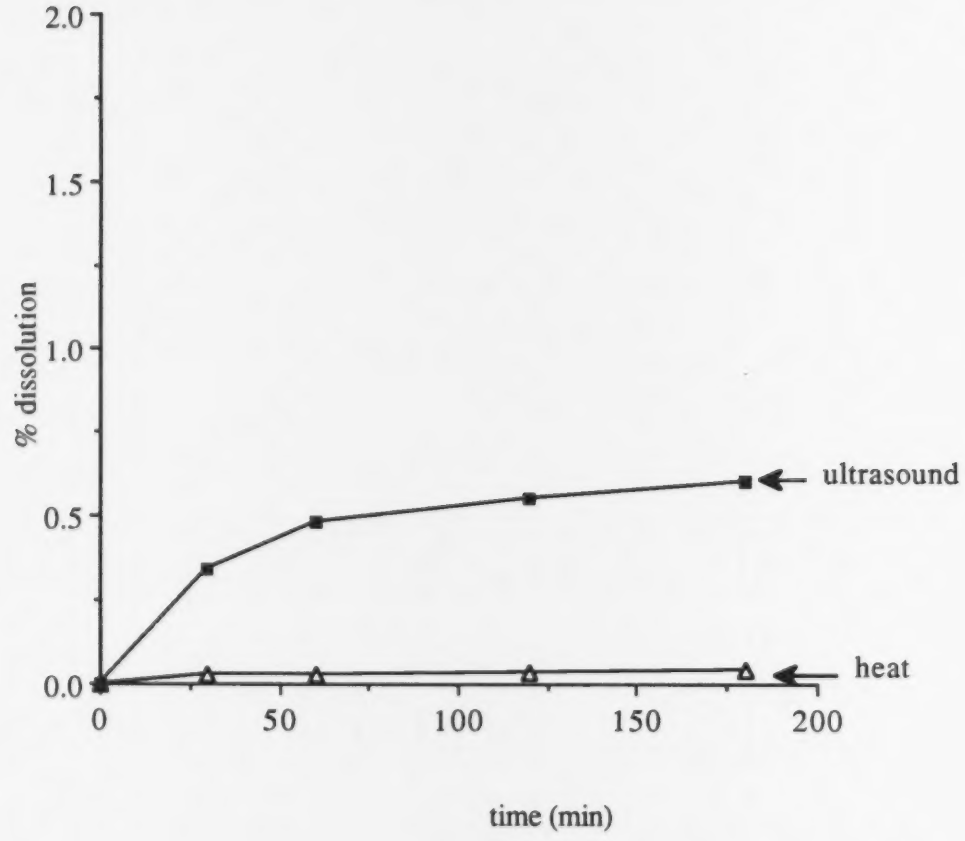


Figure 4.4 Effect of Heat and Sonication on Fraction of Iron Released From Hemin in Water as a Function of Sonication Time. Conditions: 100 mg hemin in 50 mL of water, either sonicated at 600W at a probe-vessel bottom distance of 8 cm, or heated at 100 °C on a hot plate.

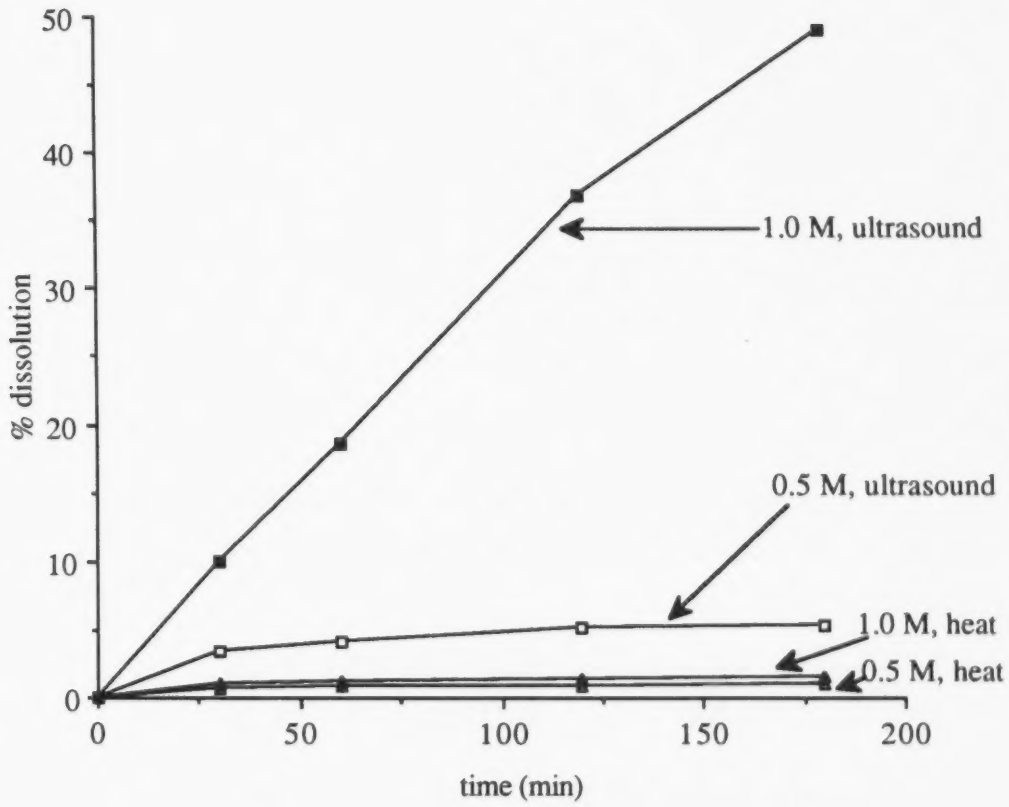


Figure 4.5 Effect of Heat and Ultrasound Treatment on Dissolution of Hemin as a Function of Time in Dilute Sulfuric Acid. Sonication was performed on 100-mg portions of hemin in 50-mL volumes at a power of 600W and a probe-vessel bottom distance of 8 cm.

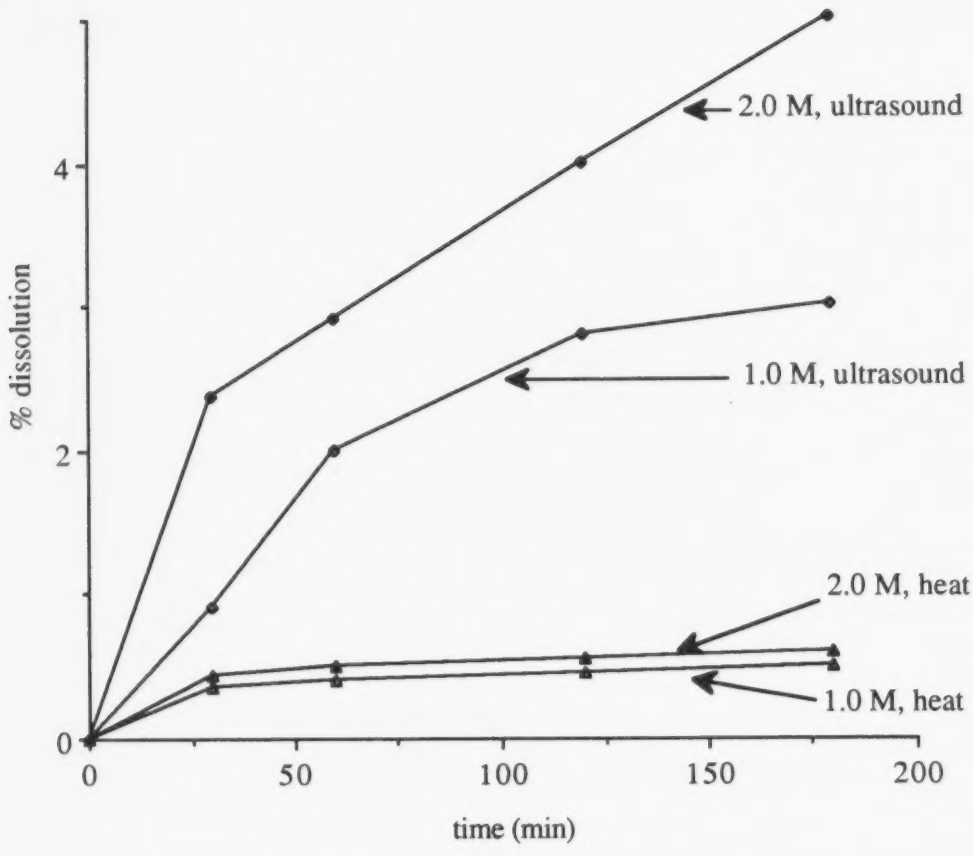


Figure 4.6 Effect of Heat and Ultrasound Treatment on Dissolution of Hemin as a Function of Time in Dilute Hydrochloric Acid. Sonication was performed on 100-mg portions of hemin in 50-mL volumes at a power of 600W and a probe-vessel bottom distance of 8 cm.

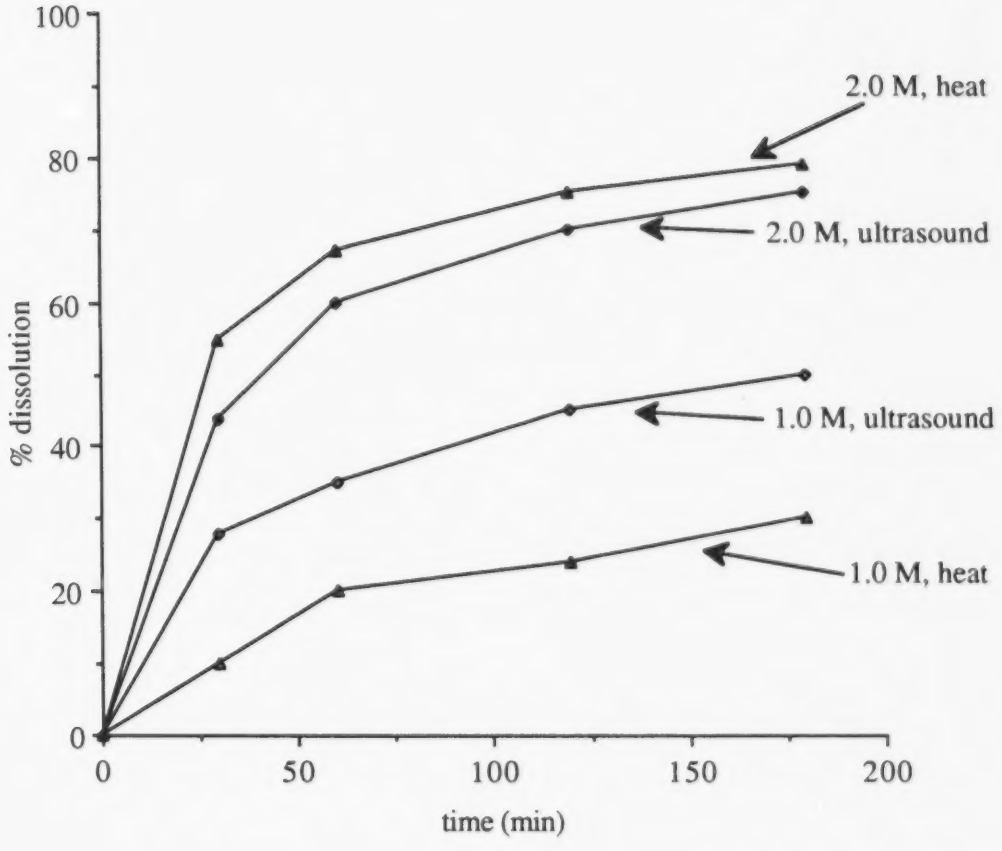


Figure 4.7 Effect of Heat and Ultrasound Treatment on Dissolution of Hemin as a Function of Time in Dilute Nitric Acid. Sonication was performed on 100-mg portions of hemin in 50-mL volumes at a power of 600W and a probe-vessel bottom distance of 8 cm.

Of the three mineral acids studied, HCl was found to have the least effect on the dissolution of hemin. The results on heating in 1.0 or 2.0 M HCl were no different from those obtained on sonication in water (less than 0.5% of iron was released). Upon sonication in HCl, however, a significant increase in the Fe signal to about 5% of the total present in the hemin was seen in 3 hrs.

Sonication in 1.0 M solutions of sulfuric acid led to 50% release of iron in 3 hrs, compared to only 5% in 0.5 M sulfuric acid. Sonication again led to significantly higher dissolution rates than heating; for heating, iron release was unaffected by the concentration of H_2SO_4.

The most extensive release of iron was observed in nitric acid. Regardless of whether heat or ultrasound was used to speed up dissolution, 2 M solutions of this acid resulted in the release of approximately 80% of the iron in 3 hrs. In 2 M HNO_3, the expected trend was reversed, i.e., sonication actually gave lower values than heating.

4.3.2.5 Effect of Heat and Ultrasound on Hemin in H_2O_2

Decomposition of organic compounds for elemental analysis by use of hydrogen peroxide in place of mineral acids leads to solutions with lower salt content; such solutions are easier to aspirate, and reduce build-up of residue in the ICP torch. In addition, excess hydrogen peroxide decomposes cleanly to produce water and oxygen[221]; it has a high dielectric constant and is thus a good electrolytic solvent. Even though the results in the previous section indicate that 2 M solutions of nitric acid provide the best results, we wanted a reagent composition for the dissolution of biological compounds that used minimal amounts of mineral acid.

The results shown in Figure 4.4 indicate that free Fe released upon decomposition of hemin by ultrasound-produced free radicals and peroxide in

water is significantly higher than that obtained under hot plate conditions. The problem with this approach is that 100% dissolution was not achieved even after 180 minutes of sonication. The results shown in Figure 3.9 indicate that the quantity of oxidant generated is sufficient to decompose many organic materials at mg/L levels, but not quite adequate to achieve total decomposition of 100 mg of hemin in 50 mL of solvent. Therefore hydrogen peroxide was added to provide more oxidizing capacity.

To study the effect of ultrasound and heat on the decomposition of hemin with added hydrogen peroxide, 100-mg portions of hemin were weighed into 100-mL beakers and 50 mL of either 0.5% or 2.5% H_2O_2 added. The beaker contents were then heated on a hot plate at 100 °C for various times. Hemin samples for sonication were prepared in a similar manner. Blanks were prepared and run through the same procedures as the samples. The results of this investigation are shown in Figure 4.8. Decomposition on a hot plate was found to be slower and less complete than sonication. Upon sonication in 2.5% H_2O_2, complete dissolution was achieved within 3 hours.

4.3.2.6 Effect of Heat and Ultrasound on Hemin in Acidic Solutions of Hydrogen Peroxide

The oxidizing power of hydrogen peroxide is strongly dependent on the pH of the solution, as shown by the following equations:

$$H_2O_2 + 2H^+ + 2e^- \rightleftharpoons 2H_2O$$

$$E = 1.763 - \frac{RT}{nF} \ln \frac{1}{[H^+]^2[H_2O_2]}$$

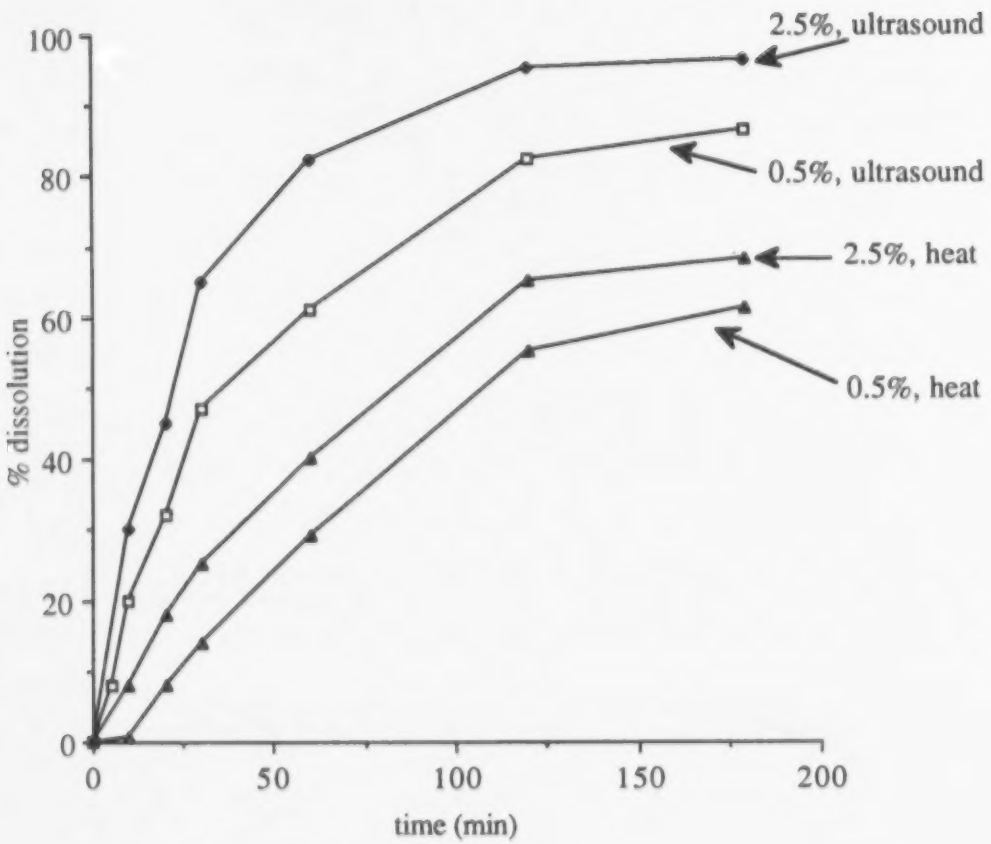


Figure 4.8 Effect of Heat and Ultrasound on Release of Fe from Hemin into Solution at Various Concentrations of H_2O_2.

To study the effect of acid on dissolution rates of hemin in hydrogen peroxide, sulfuric acid was added to the samples prior to sonication. Since optimal cavitation was achieved in 0.5 M H_2SO_4 (Figure 3.17), sonication was first conducted in a solution of this composition. The results, shown in Figure 4.9, indicate that sonication in this solvent mixture enhances dissolution rates appreciably when compared to sonication in the absence of acid. Upon sonication in 0.5% H_2O_2/0.5 M H_2SO_4 mixtures, complete dissolution with no visible traces of solid hemin was achieved within 1 hr. By increasing the peroxide levels five-fold to 2.5%, complete dissolution was achieved within 20 min. Dissolution by heating, on the other hand, was very slow in both 0.5 and 2.5% H_2O_2. The rates of dissolution of hemin under ultrasound thus approach those attainable by microwave digestion, but smaller quantities of reagents can be used. Peroxide concentrations greater than 2.5% in 0.5 M H_2SO_4 save little time because at higher peroxide levels gas evolution during sonication becomes so violent that sample is lost unless the ultrasound power level is reduced to about 20% of maximum. Since there is a direct relationship between ultrasound intensity and sonochemical effects (Figure 3.9), the rate of sample decomposition in ultrasound is significantly reduced when the power delivered to the digestion vessel is reduced.

We have also found that release of iron by sonication of hemin in 2.5% H_2O_2/1.0 M HCl or in 2.5% H_2O_2/1.0 M HNO_3 to be very slow. Complete dissolution in these solvent mixtures was not attained within the 3-hr window used in this work. Sonication in the latter solvent mixture is also not recommended because of the evolution of highly corrosive NO_2.

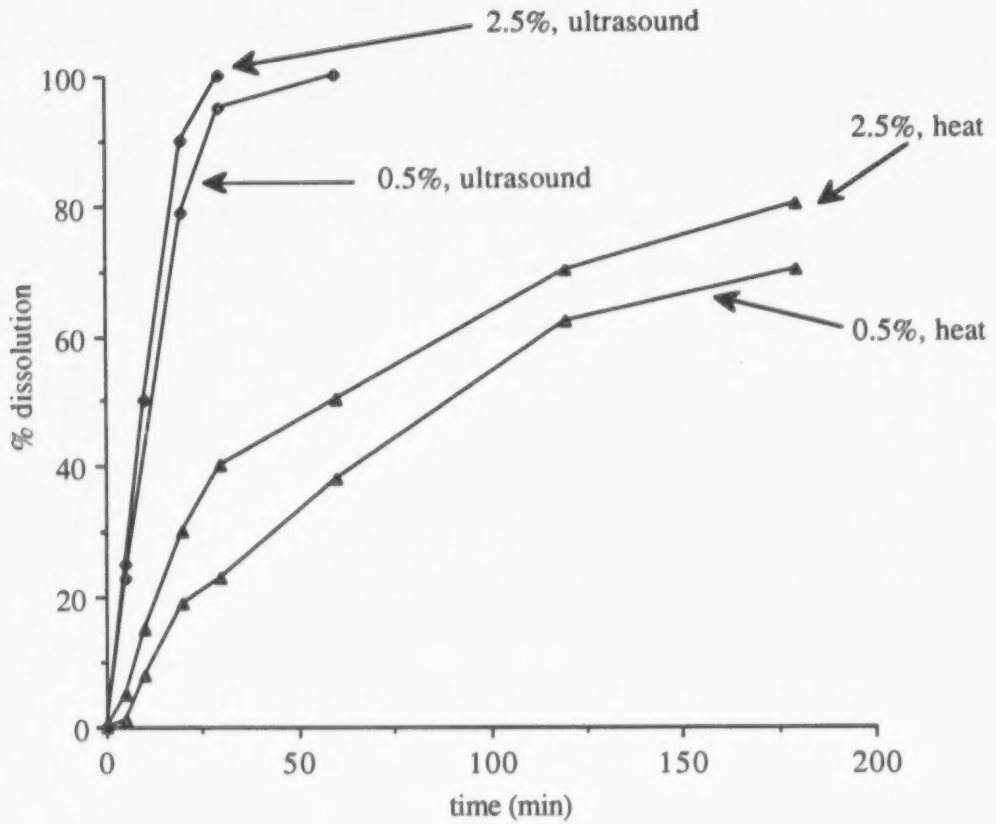


Figure 4.9 Effect of Heat and Ultrasound on Release of Fe from Hemin into Solution at Various Concentrations of H_2O_2 in 0.5 M H_2SO_4.

4.3.2.7 Summary of Results of Hemin Study

The production of oxidising species, and the generation of large areas of fresh sample surface, by ultrasound enhances the release of iron from hemin in sonicated water. The quantities of oxidant produced by sonication are not sufficient to completely dissolve 100-mg portions of hemin, so addition of hydrogen peroxide to boost the oxidising capacity was found useful. The rate of iron release in 0.5 M H_2SO_4 increased with H_2O_2 concentration; however at higher levels of H_2O_2, sample loss through foaming was a problem.

Though nitric acid is a stronger oxidising agent than sulfuric acid under conventional conditions, the release of iron from hemin was more rapid in 0.5 M H_2SO_4 than in 1.0 M HNO_3. This is probably a result of decomposition of HNO_3 by sonication as indicated by the copious amounts of nitrogen dioxide evolved from the digestion mixture and the amount of unreacted hemin remaining even after 3 hours. In HCl, chlorine gas is generated and a considerable fraction of the hemin also remains undigested. The generation of Cl_2 parallels the formation of I_2 from sonicated KI solutions. These side reactions reduce the effectiveness of the reagent oxidants.

In conclusion, the optimum solvent for complete dissolution of hemin was established to be 2.5% H_2O_2 in 0.5 M H_2SO_4. This solvent composition forms the starting point for the development of an ultrasound procedure for the dissolution of biological samples. This work is discussed in the next section.

4.3.3 Application of H_2O_2/H_2SO_4 Mixtures as Solvent for the Decomposition of Biological Samples by Sonication

The hemin experiments described above indicate that it is highly likely that naturally occurring metal-containing complexes such as the chlorophylls, hemoglobin, and vitamin B_{12}, as well as many other organic molecules found in

biological tissues, may be readily decomposed by ultrasound provided H_2O_2 and H_2SO_4 are present. In order to investigate the rates and extent of dissolution of biological samples by this system, two certified biological reference materials were analyzed for several metals. NRCC TORT-1, lobster hepatopancreas, was chosen on the basis of availability and because it has been shown to have a high degree of homogeneity for a large number of trace elements.[222] In addition, it is not easily dissolved by the usual methods.

NIST SRM 1638a, rice flour, was chosen because it contains cellulose, a chemically resistant polymer.

Bottles of these reference materials were tumbled end over end for two hours, and the material dried overnight in an oven at 85 °C. The dried material was stored in a desiccator when not in use.

4.3.3.1 Procedure and Results for TORT-1, Lobster Tissue CRM

100-mg portions of TORT-1 were accurately weighed into a sonication vessel, 50 mL of NANOpure@ water added, then 1 mL of 30% H_2O_2 and 2 mL of concentrated sulfuric acid. The mixture was sonicated at full power for a specified time, then transferred to a 100-mL volumetric flask and diluted to volume. Blanks containing reagents only were prepared in the same manner and sonicated as for the samples. Measurements were made for the trace metals Cd, Cu, Fe, Mn and Sr. Standards for each of these metals were run before and after the samples.

Figures 4.10 through 4.14 show plots of total metal concentration found in solution versus sonication time.

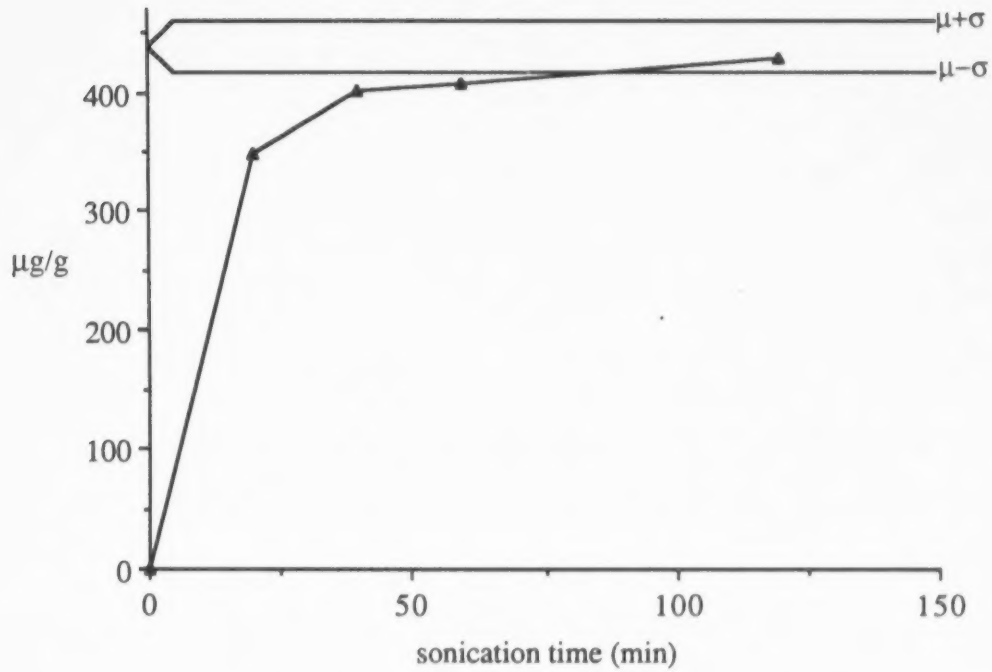


Figure 4.10. Amount of Dissolved Cu Found in Sonicated Solutions of TORT-1 as a Function of Sonication Time. μ is the certified value (439 μg/g) and σ the standard deviation (22 μg/g) at the 95% confidence level. Emission signals were measured at 324.8 nm.

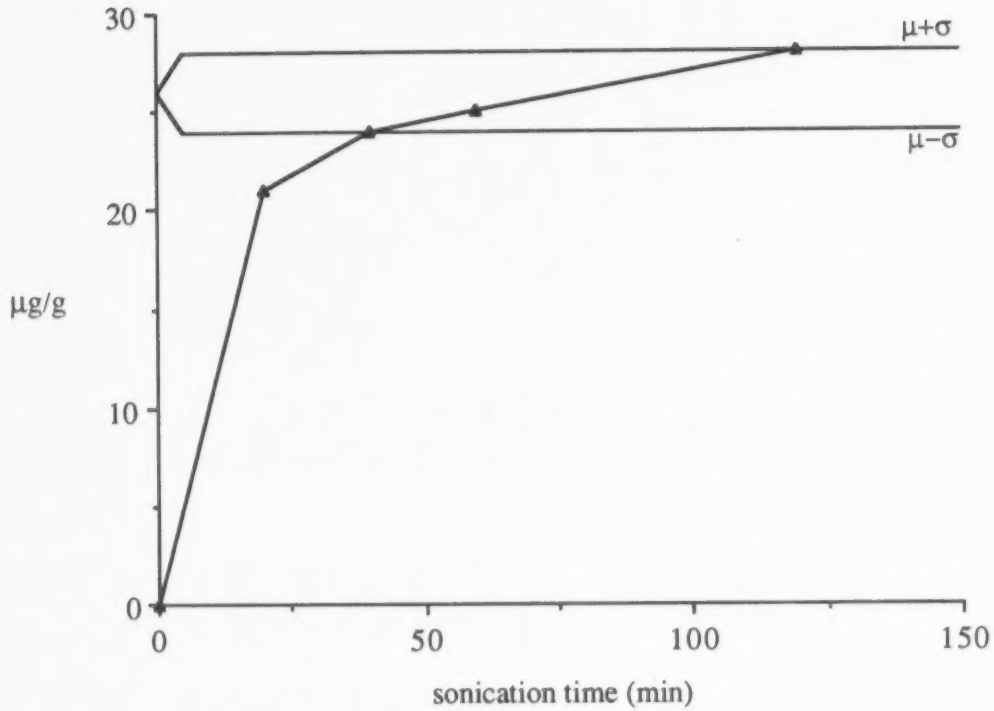


Figure 4.11 Amount of Dissolved Cd Found in Sonicated Solutions of TORT-1 as a Function of Sonication Time. μ is the certified value (26.3 μg/g) and σ the standard deviation (2.1 μg/g) at the 95% confidence level. Emission signals were measured at 226.5 nm.

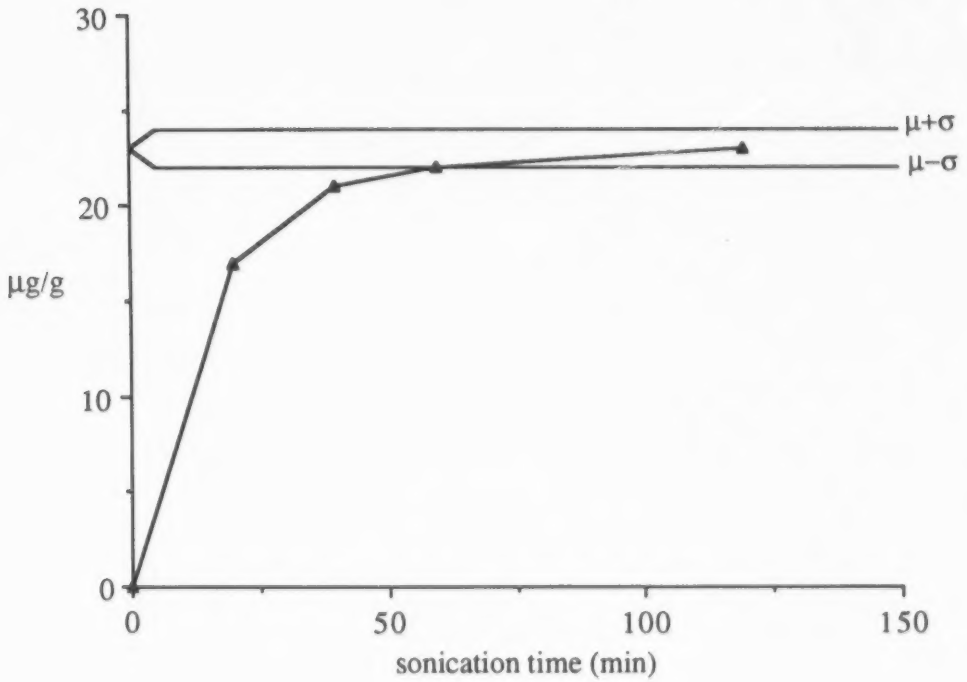


Figure 4.12 Amount of Dissolved Mn Found in Sonicated Solutions of TORT-1 as a Function of Sonication Time. μ is the certified value (23.4 μg/g) and σ the standard deviation (1.0 μg/g) at the 95% confidence level. Emission signals were measured at 257.6 nm.

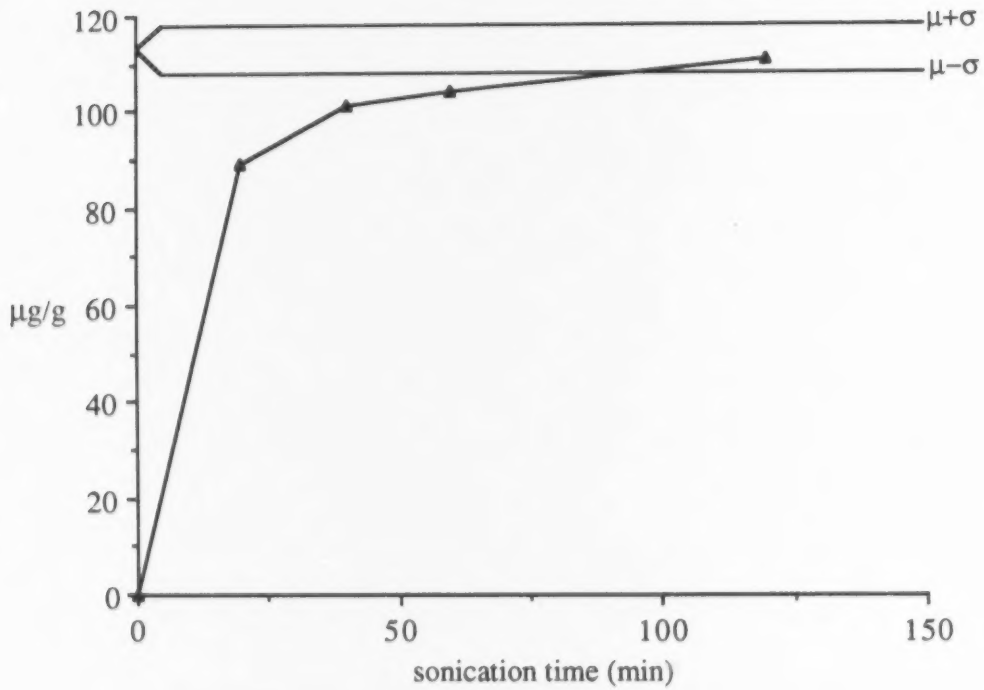


Figure 4.13. Amount of Dissolved Sr Found in Sonicated Solutions of TORT-1 as a Function of Sonication Time. µ is the certified value (113 µg/g) and σ the standard deviation (5 µg/g) at the 95% confidence level. Emission signals were measured at 407.8 nm.

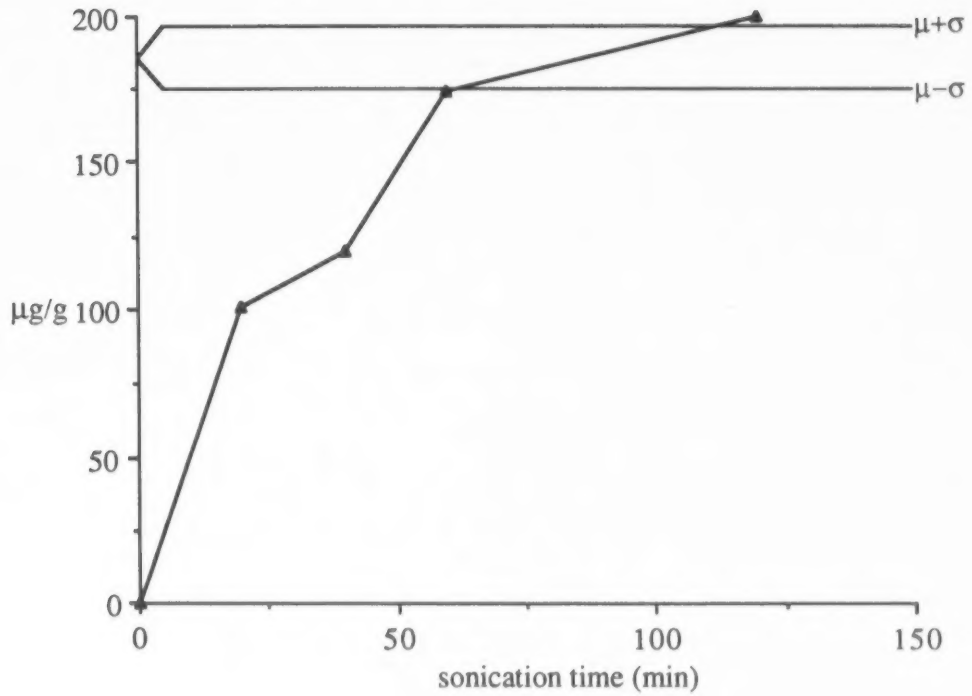


Figure 4.14. Amount of Dissolved Fe Found in Sonicated Solutions of TORT-1 as a Function of Sonication Time. µ is the certified value (186 µg/g) and σ the standard deviation (11 µg/g) at the 95% confidence level. Emission signals were measured at 259.9 nm.

Complete dissolution in this solvent mixture to yield clear solutions with no trace of solid residue was achieved within 1 hour, and the values found for 1-hr sonication agree satisfactorily with the certified values for all five elements, considering that only a single analysis was done at the 1-hr time. At sonication times less than 1 hr, filtration was required prior to measurement because some solid residue still remained. Sonication for two hours gave small increases in the amounts of metals found and generally gave results falling closer to the certification values.

To study the effect of hydrogen peroxide concentration on dissolution of TORT-1 in 0.5 M H_2SO_4, the experiment was repeated using 10 mL of 30% H_2O_2 instead of 1 mL to give a final concentration of about 5%. The results for Cu, shown in Figure 4.15, indicate a marked improvement in the rate of dissolution. Complete dissolution of a 100-mg portion of TORT-1 was achieved in less than 25 minutes, and the analytical values for copper were in good agreement with the certified values. This result confirms that control of the H_2O_2 concentration provides a handle by which dissolution rates can be controlled (Figure 4.9); by increasing the level of peroxide from 0.5% to 5%, the time taken to achieve 100% dissolution was decreased from 60 minutes to less than 25 minutes. As in the case of hemin (section 4.3.2.6), the risk of sample loss by foaming is enhanced by sonication in 5% H_2O_2; a trade-off between dissolution rate and possible loss of sample must be made accordingly.

4.3.3.2 Procedure and Results for NIST 1638a, a Rice Flour SRM

To evaluate the applicability of the procedures developed above to botanical samples, 100-mg portions of NIST SRM 1638a (Rice Flour) were accurately weighed into a digestion vessel, 50 mL of NANOpure@ water added, then 1 mL of 30% H_2O_2 and 2 mL of concentrated sulfuric acid.

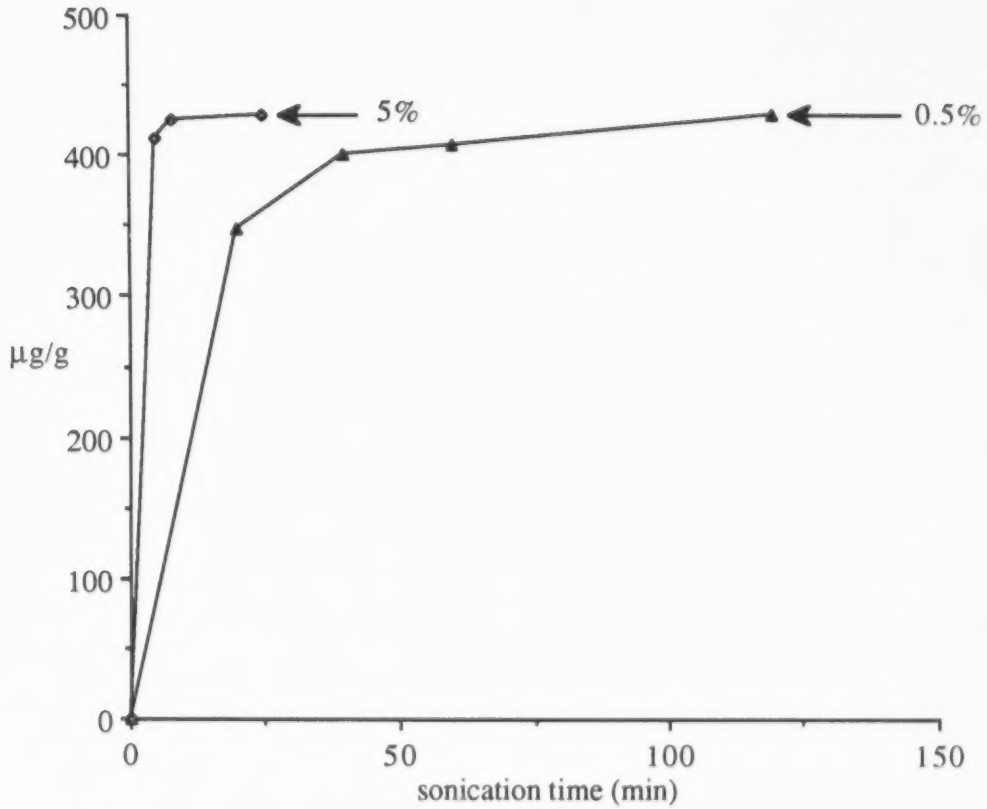


Figure 4.15. Amount of Cu Released into Solution During Sonication of TORT-1 in 0.5 M H_2SO_4 as a Function of Sonication Time and Concentration of H_2O_2.

Each mixture was sonicated at full power for 1 hr, and the resulting solution diluted to 100 mL. Blanks were prepared by sonicating the same quantities of reagents, minus the sample, at the same power and for the same time. Mn was the only essential trace metal that could be measured because the other elements were below the detection limits (less than 1ppm) of the ICP-OES analytical procedures used here. However, Ca and Mg could be determined.

Table 4.4 shows the recovery of these elements after sonication for 1 hr. There was no visible residue remaining after this time. This is consistent with literature reports of hyaluronic polymer degradation in water using 150W ultrasound waves, where the molecular weight of the polymer was found to have decreased by 50% after a 5-min sonication period.[194]

The experimental result for Mn is in good agreement with the certified value, but results for Ca and Mg are somewhat lower than the certified values.

4.3.3.3 Summary of Results Obtained for TORT-1 and Rice Flour

The ultrasound dissolution procedure for 100-mg portions of TORT-1 in 0.5 M H_2SO_4/0.5% H_2O_2 mixtures seems to work quite well. Increasing the concentration of H_2O_2 to 5% speeded the process markedly, though loss by foaming was a potential problem. However, the Ca and Mg results obtained for the rice flour (SRM 1638a) were low. The Ca might be precipiting as the insoluble sulfate, or the H_2O_2 strength is too low for complete dissolution to be achieved within 60 min. A concentration of 2.5% H_2O_2 was therefore used in all subsequent work, and, as will be seen in Chapters V and VI, was found satisfactory for most of the applications studied.

Table 4.4 Comparison of Experimental Results with Certified Values for Three Elements in NIST SRM 1638a, Rice Flour. Experimental results were obtained by ICP analysis of 4 samples, each sonicated in 0.5M H_2SO_4/0.5% H_2O_2 for 60 min at 600W power. Experimental uncertainties are $\pm 1\ \sigma$ at the 95% confidence level, while certified values are 95% tolerance values.

element	experimental value	certified value
Mn, ppm	19 ± 1	20 ± 2
Ca[1], %	0.011 ± 0.001	0.0118 ± 0.0006
Mg[2], %	0.043 ± 0.006	0.056 ± 0.002

[1] Measurements were made at 317.9 nm.

[2] Measurements were made at 279.1 nm.

4.4. SUMMARY

The results presented in this chapter show that the proposed ultrasound scheme can accelerate sample dissolution for elemental analysis of biological samples. Conditions for enhancement of dissolution rates can be explained on the basis of the results presented in Chapters II and III, in that cavitation produces reactive intermediates in the aqueous solvent and that the solvent accessible surface area increases during sonication. Both processes occur *at the same time*.

The proposed method does not require the use of concentrated and often hazardous acids. Best results were obtained with dilute solutions of sulfuric acid (0.5 M) and hydrogen peroxide (2.5%). Advantages of use of smaller quantities of reagents include a decrease in the likelihood of contamination, as well as lower costs. Reagent savings would be offset, of course, by the cost of the ultrasonic system, but current costs of ultrapure acids for analytical use could make the break even point arrive relatively quickly.

When the ultrasound sample preparation procedure is combined with a multielement measurement technique such as ICP-OES, sample preparation and determination of many trace, minor and major elements can be performed rapidly and easily on a single test portion without separate dilution for some elements.

In the next chapter, the applicability of this procedure to a range of samples from environmental, agricultural, food and clinical sources will be investigated.

CHAPTER V

APPLICATION OF ULTRASOUND TO DISSOLUTION OF BIOLOGICAL MATERIALS FOR ELEMENTAL ANALYSIS BY ICP-OES

5.1 INTRODUCTION

5.1.1. General

In Chapter I, the problem of dissolution of solid samples prior to elemental measurement by techniques such as ICP was outlined. Mention was made that analytical chemists have spent a great deal of time developing new analytical instruments to measure sub-trace levels of elements in samples with speed, accuracy and precision. Mention was also made of a general consensus[58, 59] among the analytical community that one area in the total analytical scheme that has not made comparable progress is sample dissolution. Finally, a proposal to use ultrasound to speed up dissolution of samples prior to analytical measurement was made. This suggestion was taken up in Chapters II and III, where the effect of ultrasound on solids suspended in solutions was investigated. Ultrasound was found to erode mechanically the surface of the sample, and at the same time to form oxidants from water. In Chapter IV, advantage was taken of these findings to develop an effective strategy for sample dissolution; it was noted that dissolution of biological solids using ultrasound occurs many times faster than when a hot-plate is used. A dissolution scheme based on the use of ultrasound was subsequently developed in which the use of high concentrations of hazardous acids was avoided. In this chapter,

the procedure is applied to a range of samples from environmental, agricultural, food and clinical sciences.

5.1.2. Evaluation of Performance of Ultrasound Dissolution Scheme

To evaluate the performance of the ultrasound dissolution scheme proposed in the last chapter, we chose to study a variety of certified reference materials (CRM's). As mentioned in Chapter IV, CRM's play a major role in analytical method development by providing a convenient benchmark against which the accuracy and precision can be compared.

CRM's for elemental analysis are distributed internationally by British Standards (UK), Agriculture Canada, the National Research Council (NRC) of Canada, the National Institute of Standards and Technology (NIST, USA), the South African Bureau of Standards (SABS), the National Institute of Environmental Studies (NIES, Japan), etc. Reference materials used to evaluate the performance of the ultrasound dissolution method proposed here were obtained from NIST, NRC and NIES.

5.2 EXPERIMENTAL

5.2.1 Materials

Marine biological reference material TORT-1, prepared from acetone extracted *Lobster hepatopancreas*, was obtained from the Marine Analytical Standards Program, Division of Chemistry, National Research Council of Canada, Ottawa, Canada. Powdered mussel (*Mytilus edulis*, NIES CRM #61) and high-cadmium unpolished rice flour (NIES CRM #10c) were obtained from the National Institute for Environmental Studies, Tsukuba, Japan. Rice flour (SRM 1568a),

oyster tissue (SRM 1566), pine needles (SRM 1575), bovine liver (SRM 1577a) and citrus leaves (SRM 1572) were supplied by the National Institute of Standards and Technology (formerly the National Bureau of Standards), Gaithersburg, MD, USA. Agriculture Canada reference materials 8412 (*Zea mays* stalk) and 8435 (whole milk powder) were also obtained from NIST. Hair samples (not certified) were from the author.

5.2.2 Solutions

Ultrapure water (18.3MΩ.cm) was prepared by distillation, followed by passage through a Barnstead D4751 NANOpure@ ultrafiltration system (Dubuque, IA, USA). Standards for ICP-OES were prepared by dilution of 1000 μg/mL stock solutions of Mg, Na, K, P, Ca, Cr, Pb, As, Ni,, V, Cd, Al, Sr, Cu, Fe, Zn and Mn (Fisher Scientific Co.). Concentrated H_2SO_4 and 30% H_2O_2 were analytical grade (BDH Chemicals, Toronto, Canada).

5.2.3. Sonication Procedure

Portions of sample (dried overnight in an oven at 85 C) were weighed into sonication vessels (described previously in section 4.2.3). Approximately 50 mL of NANOpure@ water, 5 mL of 30% H_2O_2 and 2 mL concentrated H_2SO_4 were added. The vessel was then placed in the ultrasound unit (Figure 3.1) and additional water added as necessary to submerge the tip by about 3 mm (the tip to vessel bottom distance was held at 8 cm throughout. Sonication power was set initially at 20% power for 2 min to avoid sample from creeping up the probe, then increased to 100% and held there for 40 min. During this process close supervision is recommended to check for sample losses and also to occasionally wash down any solid particles that may creeping up the probe. At the end of the sonication, the probe was rinsed, and the resulting orange solutions (the colour is

likely due to the presence in sonicated solutions, of TiO_2^{2+} species[223]) diluted to 100 mL. Blanks were prepared by sonication of reagents without the sample.

5.2.4 ICP Atomic Emission Measurements

The ICP procedure and operating conditions were laid out in section 4.2.2.2. The instrument was allowed to warm up for 30 min prior to measurement (see section 5.3.1.1). For quantification, multi-element standards were run before and after the sonicated samples. The ratio of emission signal intensity between samples (after blank correction) and standards was used to determine the amount of analyte in sample.

5.3 RESULTS AND DISCUSSION

The reference materials investigated in this study are conveniently classified as environmental, agricultural, food and clinical samples. Some of these classes do overlap, because some elements such Co, Ni, V, Cr, Zn and Cu, though essential for growth and development at low concentrations (micronutrients), become toxic at high concentrations from man-made sources (industrial pollutants). In addition, some of the food samples, for example, oyster tissue, can be considered as environmental samples because these organisms are used as routine bioindicators of marine pollution.

The elements measured in the samples were the essential major and minor elements Mg, Na, K, P and Ca, the essential trace elements Ni, V, Cu, Cr, Fe, Zn and Mn, and the elements of possible environmental concern Sr, Pb, Cd, Al and As. Although essential, elements such as Co and Se were not measured because

they were present in the materials investigated at sub-ppm levels, below the detection limits of the analytical procedure used here.

It was noted in section 4.4 that increasing the levels of peroxide in the sonication mixture reduces the time required for total dissolution. However, problems associated with sample loss due to foaming arise when levels of peroxide are too high. The peroxide levels used in these applications were chosen to avoid sample loss through foaming while allowing digestion to be accomplished in a reasonable time.

5.3.1 Application to Environmental Samples

Lobster and mussel reference materials were selected to represent marine animal species which are known to bioaccumulate metals. Since these organisms constitute a significant portion of the food supply for some, they are routinely analyzed by government agencies such as the United States Food and Drug Administration (FDA) and Health Canada's Food Research Center for toxic elements in order to protect the public from consuming toxic amounts.

Powdered pine needle and high-level Cd unpolished rice flour samples were selected to represent vegetation grown near sources of urban pollution.

5.3.1.1 Dissolution of Powdered Mussel Tissue and the Effect of Sample Weight

Mussels (*mytilus edulis*) are used extensively as sentinel organisms to monitor coastal water pollution by heavy metals. This reference material was prepared by grinding whole soft parts of the organism under cryogenic conditions. The average particle diameter is 177 μm.[224]

The results shown in Table 5.1 compare the experimental values for a variety of elements found in the ultrasound digests of NIES #6 to the certified values.

Table 5.1 Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values[224] for NIES #6, Powdered Mussels. All concentrations are in μg/g, unless stated otherwise. nc means not certified, nd means not detected, • means value not certified, and is to be used as reference only. Uncertainties are ± 1σ at the 95% confidence level for experimental results, and 95% tolerance levels for certified values. n=4 for both sample sizes.

element	experimental value (100-mg portions)	experimental value (800-mg portions)	certified value
Sr	17±5	17±2	17•
Cu	nd	5±3	4.9±0.3
Mn	15±3	15±2	16.3±1.2
Cd	nd	0.8±0.5	0.82±0.03
Fe	103±42	103±9	158±8
Pb	nd	0.6±0.3	0.91±0.04
As	11±3	nd	9.2±0.5
Ni	nd	nd	0.93±0.06
Zn	106±3	101±2	106±6
V	nd	nd	nc
Al	nd	nd	220•
Cr	nd	nd	0.63±0.07
Ca(wt. %)	0.13±0.02	0.14±0.03	0.13±0.01
Na(wt. %)	0.96±0.09	1.01±0.05	1.00±0.03
Mg(wt. %)	0.21±0.05	0.19±0.03	0.21±0.01
K(wt. %)	0.56±0.09	0.48±0.06	0.54±0.02
P(wt. %)	0.8±0.2	0.7±0.2	0.77•

For 100-mg portions of sample, complete dissolution of the solid material was achieved, and the following trace elements were detectable: Fe, Zn, Mn, and Sr. The agreement between experimental and certified values was good for Zn, Mn and Sr, but was not so good for Fe.

In these digests, Pb, Cd and Cu, although present in the material at μg/g levels, were not detected. The problem seems to arise from the sample signal relative to the blank signal. Figure 5.1 shows a plot of emission signal intensity for copper in several reference materials as a function of sampling time for these signals on the ICP. In this plot, NANOpure@ water, used to establish the background signal, was sampled every 30 sec. Intergration time for these signals was 10 sec. The plot shows that stability of the signal is achieved after a 30-min warm-up period, after which the signal becomes constant (this is the reason why measurements were taken after the 30 min warm-up time for the instrument, see section 5.2.4). For Cu, the signal from the 100-mg mussel samples was not significantly different from the blank. An attempt was made to improve the signal relative to the blank by increasing the sample weight to 800 mg. Even though the volume of solvent mixture was kept the same, very little residue remained after the sonication procedure. After filtration, the signal for Cu, shown in Figure 5.1, in the 800 mg sample was enhanced, and the experimental result for Cu, shown in Table 5.1, was found to be in agreement with the certified value. A similar trend in the signal intensity was observed for Cd (Figure 5.2), Zn (Figure 5.3), Sr (Figure 5.4), Mn (Figure 5.5) and Fe (Figure 5.6).

Because some residue remained after sonication, it is anticipated that some elements may not have been completely solubilized. This is the case with Zn, where even though the 800-mg sample signal is higher than the in the 100-mg sample, the amount of Zn found in the 800-mg samples was actually lower than

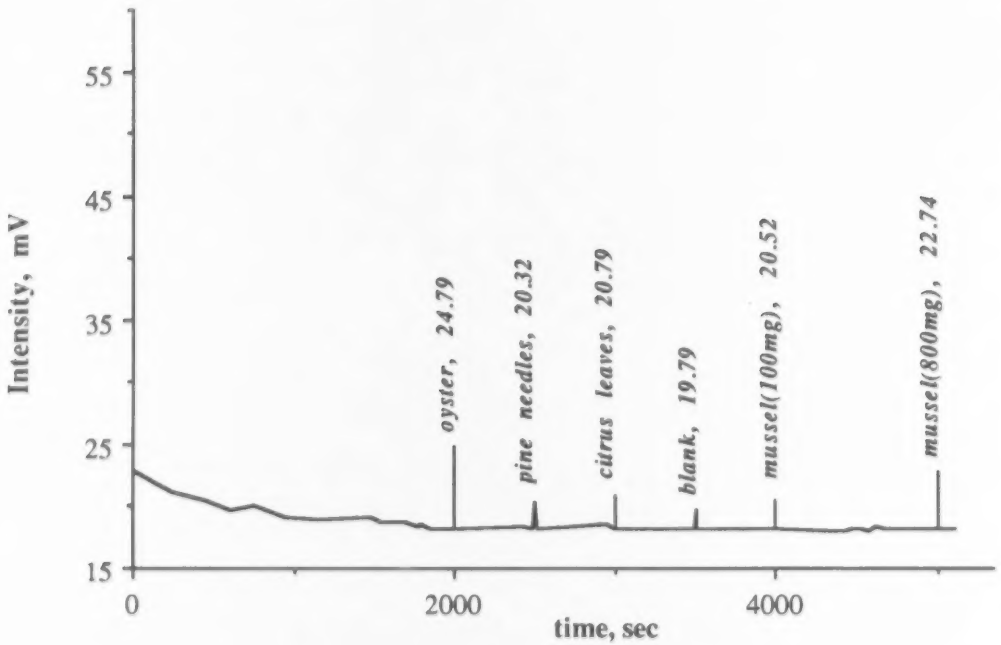


Figure 5.1 ICP-OES Emission Signals for Cu in Several Reference Materials.

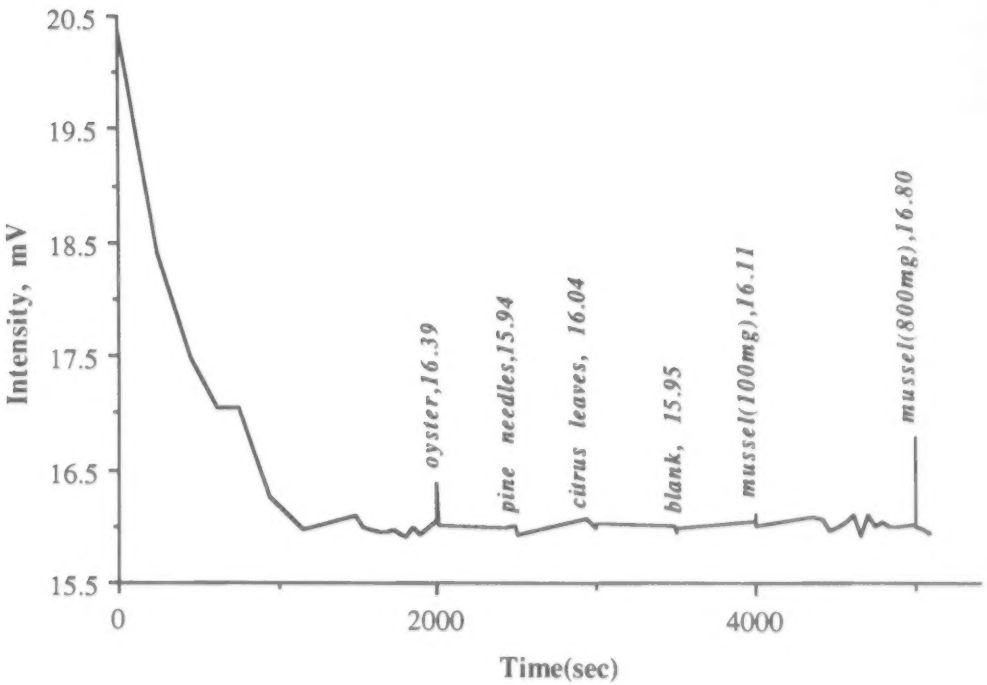


Figure 5.2 ICP-OES Emission Signals for Cd in Several Reference Materials.

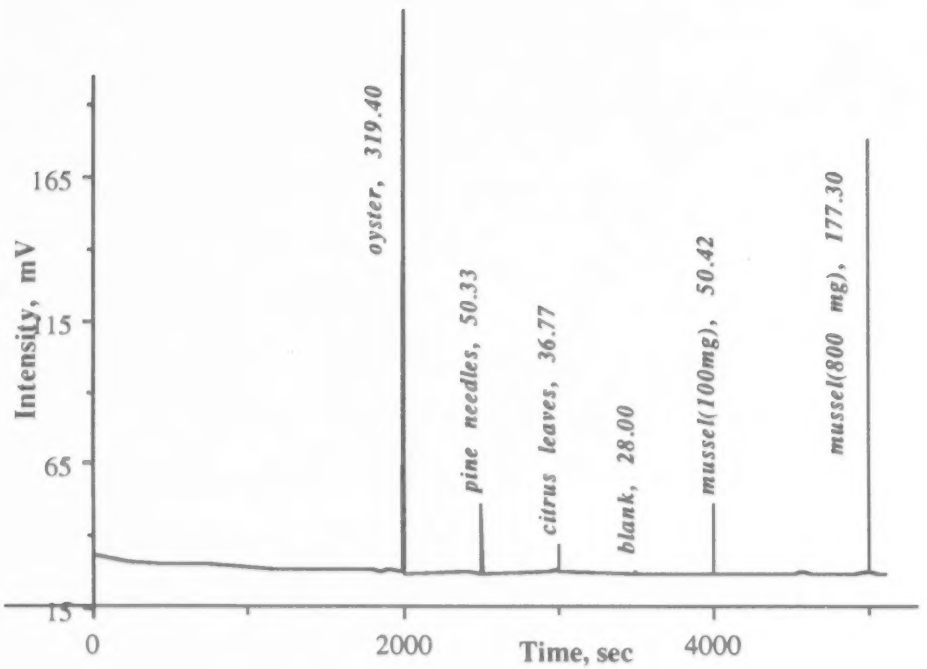


Figure 5.3 ICP-OES Emission Signals for Zn in Several Reference Materials.

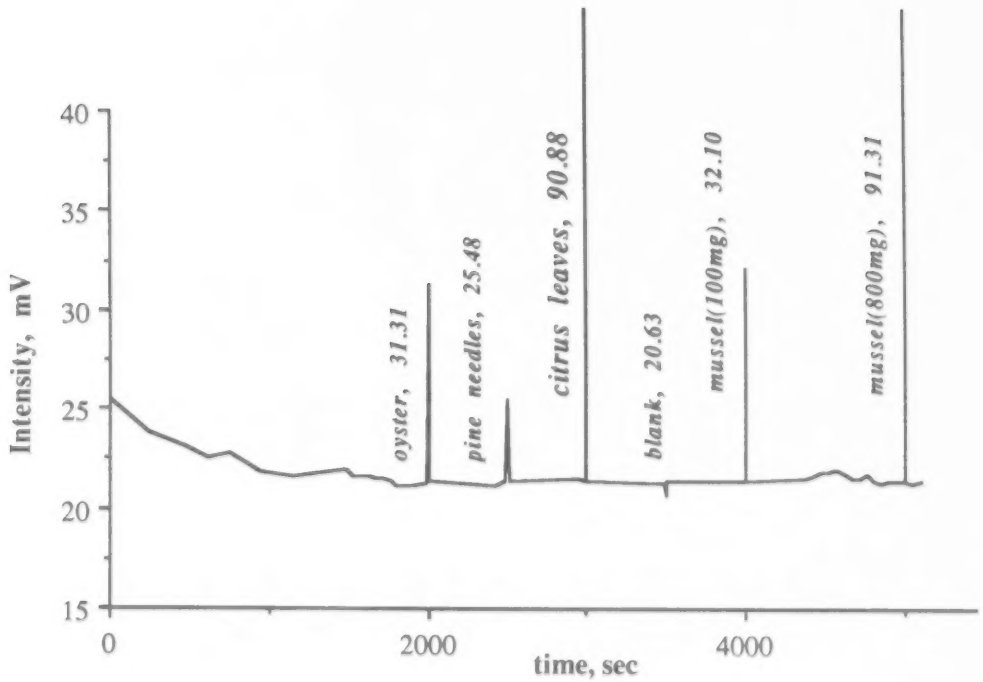


Figure 5.4 ICP-OES Emission Signals for Sr in Several Reference Materials.

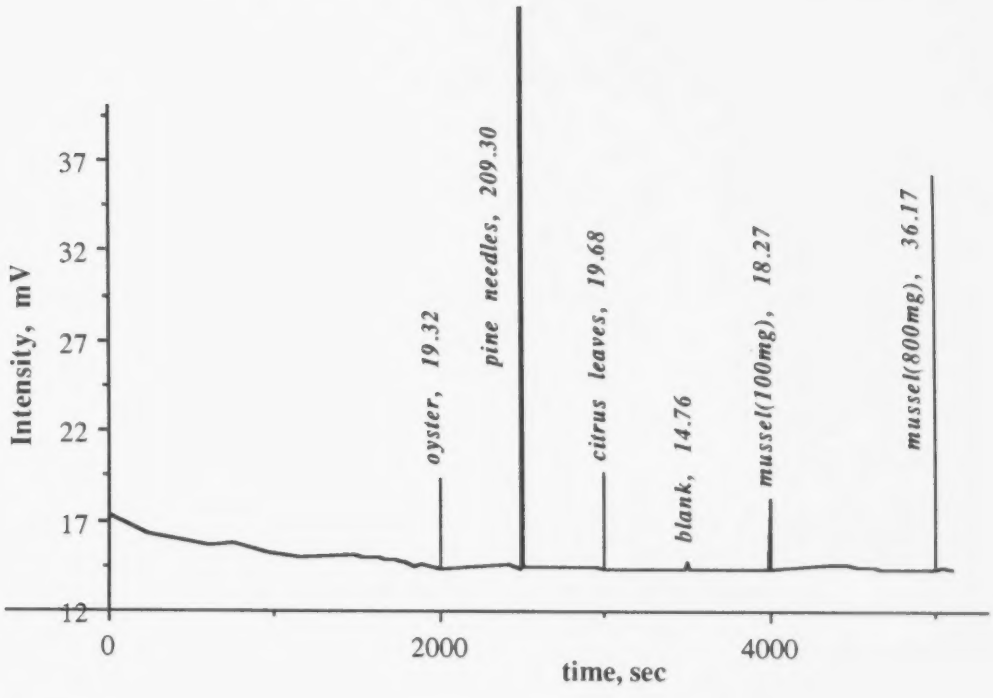


Figure 5.5 ICP-OES Emission Signals for Mn in Several Reference Materials.

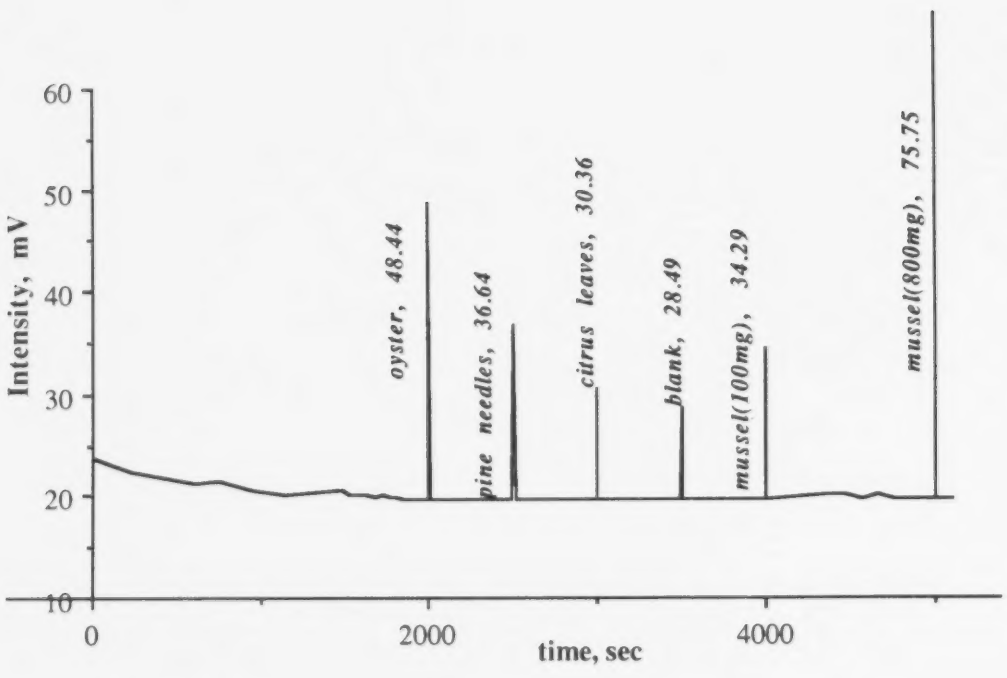


Figure 5.6 ICP-OES Emission Signals for Fe in Several Reference Materials.

the amount found in the 100-mg samples. In the case of As, no signal was detected in the 800-mg portions. The reason for this is not understood.

For 800-mg portions, the result for Fe is 55 μg/g below the certified value, but in good agreement with the results for the 100-mg portions. Aluminum, though present in the material in substantial amounts, could not be quantified. The reason for these observations is thought to be contamination from the probe itself, since a large signal from the blank subtracted from a sample signal during correction gives rise to errors. This is discussed in more detail in Chapter VI.

For the major and minor elements (Mg, Ca, Na, K, P), the signals were generally many times higher than the blanks, and values for all of these elements agreed well with certified values for 100-mg portions. For 800-mg portions, however, the result for K was somewhat low, indicating that a small but significant amount remained undissolved. A small quantity of residue was observed after sonication of the 800-mg portions by the procedure described. For these reasons sample weights of 250 mg were used for all subsequent analyses reported here. Complete dissolution of 250-mg portions of all zoological and botanical samples studied was achieved within 40 minutes, so filtration did not need to be incorporated in the dissolution procedure.

5.3.1.2 Dissolution of Powdered Lobster Tissue

TORT-1, lobster hepatopancreas, was chosen for this investigation because of the relatively high levels of trace metals present. The material was prepared by from edible-grade lobster tomalley.[222]

In Chapter IV, 100-mg portions of this material were used to optimize conditions for complete solubilization. In this investigation, 250-mg portions were used; both minor and major elements were determined without further dilution of the samples. The results, shown in Table 5.2, compare the

Table 5.2 Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values for TORT-1, Lobster Hepatopancreas.[222] All concentrations are in μg/g, unless stated otherwise. Uncertainties are ±1σ at the 95% confidence level for experimental results, and 95% tolerance levels for certified values. n=8 except for Ca (n=3).

element	experimental result	certified value
Sr	118±4	113±5
Cu	438±5	438±22
Mn	24.5±0.4	23.4±1.0
Cd	27±4	26.3±2.1
Fe	212±8	186±11
Pb	17±8	10.4±2.0
As	31±4	24.6±2.2
Ni	not detected	2.3±0.3
Cr	not detected	2.4±0.6
V	not detected	1.4±0.3
Zn	177±7	177±10
Ca (wt. %)	0.94±0.06	0.895±0.058

experimental values for a variety of elements found in the digests of TORT-1 to the certified values. For the trace metals determinable by the ICP procedure, good agreement between experimental values and certified values was found for Cu, Zn, and Mn. Among the non-essential trace elements, satisfactory agreement was found for Sr, Cd, As and Pb, although the uncertainty in the results for As and Pb was rather high. In contrast to the mussel sample results, the experimental values for Fe are high rather than low. Even though Cr and V are present in the material at levels above 1 ppm, they were not detected in the sonicated solutions, where sample signals were lower than the blank signals. Contamination from the probe was suspected; this was subsequently investigated and is discussed in Chapter VI. For calcium, good agreement was found between experimental and certified values.

5.3.1.3 Dissolution of Pine Needles

Pine needle samples were provided as a brown powder (<250 μm diameter) prepared by grinding, mixing and sieving of the plant material.[225] Dissolution and analysis of 250-mg portions of the material by the same procedure as for the preceding samples gave the results shown in Table 5.3. Fair agreement between experimental and certified values was found for Sr, Mn, Cu and Zn. The results for Mn and Sr are somewhat lower than the certified values and the uncertainties in the experimental values for Cu and Pb are rather high. Cd, Ni, V, Cr, Na and As were not detected. Values for Fe and Al are quite low, probably because of high blanks for these elements.

Among the major and minor elements, good results were obtained for K, Mg and Ca. P could not be quantified; here again the sample signals were lower than the blank signals.

Table 5.3 Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values for SRM #1575, Pine Needles.[225] All concentrations are in μg/g, unless stated otherwise. • means value is not certified, and is to be used as reference only. Uncertainties are ±1σ at the 95% confidence level for experimental results (n=4), and 95% tolerance levels for certified values.

element	experimental result	certified value
Sr	4.2±0.3	4.8±0.2
Cu	3.7±1.8	3.0±0.3
Mn	648±6	675±15
Cd (ppb)	not detected	< 500•
Fe	78±14	200±10
Pb	18±11	10.8±0.5
As (ppb)	not detected	210±40
Ni	not detected	3.5•
Al	31±14	545±30
V	not detected	not certified
Na	not detected	not certified
Zn	60±3	67±9
Cr	not detected	2.6±0.2
Mg	1179±224	1250±170
K	3794±232	3700±200
P	not detected	1200±200
Ca	4100±160	4100±200

5.3.1.4. Dissolution of High-Cadmium Unpolished Rice Flour

The determination of Cd in rice is of major importance in areas where contamination of soil has led to diseases like "itai-itai". This condition has been observed to be prevalent among Japanese who consume large amounts of rice grown in contaminated areas. The Japanese reference material NIES #10c, a high-cadmium unpolished rice flour,[226] was analyzed in this work to assess the validity of the proposed ultrasound dissolution technique on Cd analyses in environmental plant materials.

For this study, a standard additions procedure was adopted whereby, after dissolution of 250-mg portions of the material and dilution to 100-mL, 20-mL aliquots of the solution were taken and spiked with 0, 20 and 40 μL of a 10 ppm Cd standard. These solutions were then aspirated into the ICP, and the signal obtained was plotted against spike volume. A least squares calculation was used to locate the intercept; this was used to determine the amount of Cd in the original 100-mL solution.

Since the ICP is a multi-element system, and a number of other elements in the rice flour have been determined and their certified values reported, the ICP was programmed to record data for as many elements as possible. The results of these analyses are shown in Table 5.4.

Good agreement between experimental and certified values was found for Cd, Mn, Zn, Mg and Fe, even though the uncertainty associated with the Fe result was rather high. Because of the high blank levels of P and Al, these elements were not determinable in the samples. In addition, the result obtained for Ca was significantly lower than the certified value; this may be due to precipitation of part of this element as calcium sulfate. In other materials investigated in this chapter, where Ca content was in the % rather than ppm range, this did not seem to be a problem.

Table 5.4 Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values for NIES #10c, High-Cadmium Unpolished Rice Flour.[226] All concentrations are in μg/g, unless stated otherwise. • means value is not certified, and is to be used as reference only. Uncertainties are ± 1σ at the 95% confidence level for experimental results (n=4), and 95% tolerance levels for certified values.

element	experimental result	certified value
Sr	0.182±0.006	0.2•
Cu	not detected	4.1±0.3
Mn	38±3	40.1±2
Cd	1.9±0.3	1.82±0.06
Fe	14±8	11.4±0.08
Pb	not detected	not certified
As	not detected	0.15•
Ni	not detected	0.30±0.03
Al	not detected	1.5•
V	not detected	not certified
Ca	62±11	95±2
Zn	22±3	23.1±0.8
Cr	not detected	0.08
Na	not detected	14.0±0.4
Mg (wt. %)	0.12±0.08	0.125±0.008
K (wt. %)	0.39±0.03	0.275±0.010
P (wt. %)	not detected	0.335±0.008

5.3.2 Application to Agricultural Samples

The mineral composition of agricultural products is of interest to agronomists, food scientists and nutritionists. The levels of elements in agricultural materials indicate the health status of plant and animal products. In this category, three agricultural materials were selected to test the ultrasound dissolution scheme proposed in chapter IV. These were NIST SRM's 1572 and 1568a (citrus leaves and rice flour, respectively), and Agriculture Canada RM 8412, corn (*Zea mays*) stalk. Many of the trace elements for which these materials are certified were not detected by the procedure used here. Therefore the results reported in this section are mainly for minor and major elements.

5.3.2.1 Dissolution of Powdered Citrus Leaves

Samples of citrus leaves were provided as a finely ground green powder. Sonication of this material first caused disappearance of the characteristic green of chlorophyll, followed by complete dissolution to yield clear solutions. The results shown in Table 5.5 compare the experimental values for a variety of elements found in the ultrasound digests of SRM 1572 to the certified values.[227]

For the trace metals determinable by the ICP procedure used here, good agreement between experimental values and certified values was found for Zn, Mn and Sr. Cd, Pb, Ni and As were not detected. Cr and Al results could not be quantified because blanks were higher than samples. The agreement was not so good for Fe, while the uncertainty in the experimental result for Cu was very high.

Of the certified minor and major elements, P could not be quantified because blank signals were higher than those for the sample. Excellent agreement was observed for Mg, K, and Ca.

Table 5.5 Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values for SRM #1572, Citrus Leaves.[227] All concentrations are in μg/g, unless stated otherwise. • means value is not certified, and is to be used as reference only. Uncertainties are ± 1σ at the 95% confidence level for experimental results (n=4), and 95% tolerance levels for certified values.

element	experimental result	certified value
Sr	106±16	100±2
Cu	not quantified	16.5±1.0
Mn	21±2	23±2
Cd	not detected	0.03±0.01
Fe	16±10	90±10
Pb (ppb)	not detected	13.3±2.4
As	not detected	3.1±0.3
Ni	not detected	0.6±0.3
Cr	not detected	0.8±0.2
V	not detected	not certified
Al	not detected	92±15
Zn	28±3	29±2
Na	187±24	160±20
Mg (wt. %)	0.58±0.02	0.58±0.03
K (wt. %)	1.87±0.03	1.82±0.06
P (wt. %)	not detected	0.13±0.02
Ca (wt. %)	3.2±0.2	3.2±0.1

5.3.2.2 Dissolution of NIST Rice Flour

In Chapter IV, 100-mg portions of NIST SRM #1568a, rice flour, were used to optimize conditions for producing complete solubilization by ultrasound. For this section of the investigation, 250-mg portions were used, and minor, major, and trace elements were determined. The results are shown in Table 5.6. Compared to NIES #10c, unpolished high-cadmium rice flour, NIST SRM #1568a contains very low quantities of Cd. As the results show, they were too low to be detected in this material.

Good agreement between experimental values and certified values was found for Cu, Zn, and Mn. Sr was not certified, but the good accuracy observed in the determination of this element in the various matrices studied so far, we judge the experimental result worth reporting. Pb and Cd were not detected; blank signals for As, V and Al were very high. Agreement between certified and experimental values for Fe was poor.

Among the major elements, good agreement was found for Ca. The results were not so good for Mg; P could not be quantified because the blank signals were higher than those of the sample.

5.2.3.3 Dissolution of Agriculture Canada Corn Stalk Reference Material

The results shown in Table 5.7 compare the experimental values for a variety of elements found in the digests of RM 8412, corn stalk, to the recommended concentration. This reference material was prepared from stalks of corn plants grown at the Central Experimental Farm, Agriculture Canada. The stalks were washed, dried at 80 °C in an air oven, then ground and sieved to a fine powder (<210 μm dia. particle size).[228] Except for slightly low results obtained for Na and K, the other analyses show fair to good agreement between recommended and experimental values.

Table 5.6 Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values for SRM #1568a, Rice Flour. All concentrations are in μg/g, unless stated otherwise. • means value is not certified, and is to be used as reference only. Uncertainties are ± 1σ at the 95% confidence level for experimental results, and 95% tolerance levels for certified values. n=4, except Mn, Ca and Mg (n=8).

element	experimental result	certified value
Sr	0.3±0.2	not certified
Cu	2.0±1.2	2.4±0.3
Mn	18.8±0.3	20.0±1.6
Cd	not detected	0.022±0.002
Fe	17±10	7.4±0.9
Pb	not detected	< 0.010•
As	not detected	0.29±0.03
Al	not detected	4.4±1.0
Cr	not detected	not certified
V	not detected	0.007•
Sb	not detected	0.0005•
Zn	17.4±0.8	19.4±0.5
Na	not detected	6.6±0.8
Mg (wt. %)	0.043±0.005	0.056±0.002
P (wt. %)	not detected	0.153±0.008
Ca (wt. %)	0.012±0.002	0.0118±0.0006

Table 5.7 Comparison of Elemental Concentrations in Sonicated Samples Measured by ICP-OES with Certified Values for Agriculture Canada Reference Material 8412, Corn (*Zea mays*) Stalk.[228] All concentrations are in μg/g. Uncertainties are ± 1σ at the 95% confidence level for experimental results (n=4), and 95% tolerance levels for certified values.

element	experimental result	certified value
Sr	10±2	12±2
Cu	10±5	8±1
Mn	16±2	15±2
Na	54±16	28±8
Fe	160±30	139±15
K (wt. %)	1.54±0.08	1.735±0.047
Zn	30±8	32±3
Ca (wt. %)	0.22±0.02	0.216±0.008
Mg (wt %)	0.155±0.008	0.160±0.007

5.3.3 Application to Food Samples

Oyster and milk powder reference materials were used to evaluate the applicability of the ultrasound dissolution scheme to food analysis. Oysters provide a representative species for sea food, being a filter organism that closely represents the environment around it and contains a variety of components to test the dissolution process. Milk powder contains appreciable amounts of proteins such as casein that are not readily solubilized by conventional means.

5.3.3.1 Dissolution of Oyster Tissue

Samples of of NIST SRM 1566, acetone-extracted oyster tissue, were supplied as a fine powder prepared by homogenization of the whole organism. The results shown in Table 5.8 compare the experimental values for a variety of elements found in the ultrasound digests to the certified values. Among the trace metals determinable by the ICP procedure, satisfactory agreement between experimental values and certified values was found for Sr, Cd, As, Fe, Cu, Zn, and Mn. Pb was not detectable as it was in the ppb range. As expected, V and Cr results were unquantifiable because of high blank signals.

Except for low results for K, all certified minor and major elements showed good agreement between certified and experimental value.

5.3.3.2 Dissolution of Whole Milk Powder

The results shown in Table 5.9 compare the experimental values for a variety of elements found in the digests of RM 8435, whole milk powder, to the reference values. Confidence limits for the recommended values were not provided by NIST, the issuer of the this Agriculture Canada reference material. The milk powder sample, though it required minimal supervision during digestion, did not completely dissolve. A thin film of fatty substances remained.

Table 5.8 Comparison of Elemental Concentrations in Sonicated Samples (n=4) Measured by ICP-OES with Certified Values for SRM #1566, Oyster Tissue.[229] All concentrations are in μg/g, unless stated otherwise. Uncertainties are ± 1σ at the 95% confidence level for experimental results, and 95% tolerance levels for certified values.

element	experimental result	certified value
Sr	10±2	10.36±0.56
Cu	67±5	63±4
Mn	16±3	17.5±1.2
Cd	4±3	3.5±0.4
Fe	202±26	195±34
Pb (ppb)	not detected	480±40
As	18±5	13.4±1.9
Ca (wt. %)	0.149±0.008	0.15±0.02
Cr	not detected	0.67±0.27
V	not detected	not certified
Mg (wt. %)	0.122±0.008	0.128±0.009
Zn	830±30	852±14
Na (wt. %)	0.5±0.1	0.51±0.03
K (wt. %)	0.81±0.01	0.969±0.005
P (wt. %)	0.8±0.2	0.778±0.012

Table 5.9 Comparison of Elemental Concentrations in Sonicated Samples (n=4) Measured by ICP-OES with Certified Values for Agriculture Canada RM 8435, Whole Milk Powder.[230] All concentrations are in μg/g, unless stated otherwise. Uncertainties are ± 1σ at the 95% confidence level for experimental results, and 95% tolerance levels for certified values.

element	experimental result	certified value
Sr	3.1±0.2	4.35
Cu	0.7±0.6	0.46
Mn	not detected	0.17
Cd	not detected	0.0002
Fe	46±16	1.8
Pb	not detected	0.11
As	not detected	0.001
Ni	not detected	0.01
Cr	not detected	0.5
V	not detected	nc
Na (wt. %)	0.37±0.01	0.356
Zn	26±5	28
Mg	732±30	814
K (wt. %)	2.00±0.05	1.363
P (wt. %)	0.28±0.03	0.78
Ca (wt. %)	0.80±0.02	0.922

In the sonicated solutions, Mn, Cd, Pb and Ni were not detected. Blank signals for Fe, As, Cr and V were higher than sample signals because of contamination either from the reagents used for dissolution, or the probe itself. Because of incomplete dissolution and high blanks, only Zn gave a value that matched the certified number.

5.3.4 Application to Clinical Samples

Two ways of removing toxic metals from the human body are through the liver and hair. Elemental analyses of these organs can provide usefull information on nutritional and toxicological status. Liver specimen banks have been set up in several locations to provide a basis for monitoring trace element uptake by humans from the environment.

5.3.4.1 Dissolution of Acetone-Extracted Bovine Liver, NIST SRM 1577a

The results in Table 5.10 compare the experimental values for a variety of elements found in the digests of NIST SRM 1577a, bovine liver, to the certified values. This reference material was prepared by removal of the gross fat, blood vessels and skin, then mixed, lyophilized and powdered in a Tornado mill.[231] Elements that could not be detected in the ultrasound digests because of their presence at sub-ppb levels in the samples were: Sr, Cd, and Pb. As for the other biological materials studied in this work, blanks for Al, P, As and V were higher than the sample signals. Because of this, quantification for these elements was not possible. Except for low results for K, all other elements in Table 5.10 showed reasonable agreement between certified and experimental values.

Table 5.10 Comparison of Elemental Concentrations in Sonicated Samples (n=4) Measured by ICP-OES with Certified Values for SRM #1577a, Bovine Liver.[231] All concentrations are in μg/g, unless stated otherwise. Uncertainties are ± 1σ at the 95% confidence level for experimental results, and 95% tolerance levels for certified values.

element	experimental result	certified value
Sr (ppb)	not detected	138±3
Cu	160±19	158±7
Mn	8±2	9.9±0.8
Cd (ppb)	not detected	440±60
Fe	140±40	194±20
Pb (ppb)	not detected	135±15
As (ppb)	not detected	47±6
Zn	125±10	123±8
Na (wt. %)	0.26±0.01	0.243±0.013
Mg	590±16	600±15
K (wt. %)	0.970±0.008	0.996±0.007
P (wt. %)	1.14±0.03	1.11±0.04
Ca	115±16	120±7

5.3.4.2 Dissolution of Human Hair

An appropriate reference material with which to compare the results not being available at the time, samples of black human scalp hair were collected from a single individual (Swazi male) during a visit to a barber shop. Hair strands were cut into pieces approximately 2 mm in length, dried in an air oven at 80 °C overnight, and approximately 50-mg test portions weighed for analysis. Sonication was performed at full power for 40 minutes in 2.5% H_2O_2/0.5 M H_2SO_4. Of all the materials investigated in this study, dissolution of human hair required the least attention. No creeping of sample up the probe was observed, and at the end of the sonication procedure, the solution was a characteristic clear yellow was obtained. ICP analysis of the digests was carried out as for the reference materials. The results are shown in Table 5.11. The data for the elements Mn, Cd, Pb, Ni and K were below the detection limits of the ICP procedure used here, and the ICP signals for the blanks were higher than that of the samples for As, Cr, V and P. As before, contamination from the reagents or, more likely, the probe was suspected. The elements Sr, Cu, Fe, Ca, Zn, Na, Mg and Al were able to be quantified. When compared to the values reported for the NIES RM Human Hair, prepared from typical Japanese male hair,[232] the values obtained are reasonable except for Al, which was found to be much higher. An analysis of this material by INAA (see Chapter VI) will suggest that the Al result found here may be biased through contamination from the probe.

Table 5.11 Elemental Concentrations in Sonicated Human Hair Samples (n=2) Measured by ICP-OES. All concentrations are in μg/g.

element	sample #1	sample #2
Sr	15	17
Cu	23	15
Mn	not detected	not detected
Cd	not detected	not detected
Fe	40	38
Pb	not detected	not detected
I	not quantified	not quantified
Br	not quantified	not quantified
Cl	not quantified	not quantified
V	not detected	not detected
Ca	3900	3720
Zn	105	113
Na	330	250
Mg	300	420
Al	1600	800

5.4 SUMMARY

The results presented in this chapter indicate that the proposed ultrasound dissolution technique shows promise for application to a wide range of biological materials. The method appears to be satisfactory for determination of most elements to the limit of detection of the ICP method used on 250-mg samples. Zoological samples were quicker to dissolve, but materials with a high fat content needed very close supervision to avoid loss from foaming. Botanical samples, on the other hand, tended to take longer to dissolve, probably because of the slow attack of cellulose, but required minimal supervision because foaming did not occur. Except for Al and P, reasonable agreement was found between certified and experimental values for elements present at higher concentrations. For those elements present at trace levels, the method showed mixed performance. Cd, Mn and Sr results were good in all cases, but V, Cr, Fe and As where detected gave poor agreement with certified values, and the Cu results had appreciable scatter. The next chapter reports on a study of these problems.

CHAPTER VI

CONTAMINATION ASSESSMENT AND DESIGN OF HORNS FOR ULTRASOUND DISSOLUTION OF BIOLOGICAL MATERIALS

6.1 GENERAL

It was shown in Chapter V that sonication using a commercial 600 W/20 kHz ultrasound probe is capable of dissolving 250-mg portions of a variety of biological materials in 0.5 M H_2SO_4/2.5% H_2O_2. Complete dissolution of many materials investigated was found to occur within 40 minutes, but blank levels, especially for Fe, As, Cr, V, Al and P, were found to be high.

The first part of the work described in this chapter reports on an investigation of the source of the high blanks. The second part describes a modification of the horn tips to reduce the magnitude of this problem.

6.2 CONTAMINATION ASSESSMENT FROM A COMMERCIAL ULTRASONIC PROBE

6.2.1 Introduction

The commercial Sonics horn described in section 2.2.1.1, originally designed for biological cell disruption, is fabricated from a titanium alloy. Since the probe is immersed directly into solution, contamination from the alloy is a potential

disadvantage. Ultrasound spatial intensity calculations using equation 4 show that the tip of the probe experiences the harshest effects of cavitation because ultrasound intensity is greatest at this point. Results of SEM studies on Ti tips, reported in Chapter II, show that the forces involved during cavitation are energetic enough to remove fragments of metal from the surface of the probe. The presence of Ti in sonicated solutions is not generally a concern because it has no known biological activity, and so knowledge of its concentration is not normally of interest. However, impurities in the titanium could be a problem.

This section describes the use of instrumental neutron activation analysis (INAA) to determine elements present in the solid Ti horn, and inductively coupled plasma optical emission spectroscopy (ICP-OES) to analyze sonicated solutions for elements that might be released from the horn into solution. Background information about ICP-OES was given in section 4.1.2.2; the concepts behind INAA are briefly outlined below.

6.2.2 Principles of INAA

When stable nuclei present in a sample are bombarded with neutrons, radioactive isotopes are formed. Decay of these radionuclides occurs either by radiation of gamma rays or by emission of one or more nuclear particles. In gamma ray spectrometry, the gamma rays emitted during decay are sorted by energy and measured to provide a fingerprint of the elements present in the sample. By counting the number of gamma photons of particular energies emitted per unit time, quantitative information about the elements present in a material can be obtained.

A nuclear reactor is a good source of neutrons. It normally generates neutrons by fission of ^{235}U. These neutrons have a continuous kinetic energy spectrum; moderators such as H_2O or D_2O are commonly used to slow them down to

thermal energies. In the work reported here, a SLOWPOKE-2 (acronym for Safe LOW POwer Kritical Experiment) nuclear reactor was used as a thermal neutron source. Detailed information concerning this nuclear reactor facility is provided in section 6.2.3.4 below.

6.2.3 Experimental

6.2.3.1 Materials and Reagents

The sonication medium was 18.3 MΩ·cm resistivity water from a Barnstead D4751 NANOpure@ ultrafiltration unit, and the sonication vessels used in this work were 100-mL Teflon beakers. Standards for calibration were prepared by dilution of 1000 μg/mL commercial stock solutions of the elements of interest (Fisher Scientific). HF, H_2SO_4 and H_2O_2 were obtained from BDH Chemicals (Toronto).

6.2.3.2 Sonication Apparatus

A 20 kHz/600 W Sonics ultrasound unit, see section 4.2.3 for details, was used for all sonication procedures described here. The probe used in this part of the study was fabricated from an unspecified titanium alloy.

6.2.3.3 Microwave Evaporation Apparatus

A commercial 700W Sears Kenmore microwave oven (Model 87751) was used to provide rapid evaporation under dust-free conditions.[233]

6.2.3.4 University of Alberta SLOWPOKE-2 Nuclear Reactor Facility

The University of Alberta SLOWPOKE-2 Reactor Facility is located in the Dentistry/Pharmacy Building. The SLOWPOKE-2 is a small pool type nuclear reactor developed by Atomic Energy of Canada Limited. The heart of the reactor

is a core, 22 cm in diameter and 23 cm high, comprised of 297 pencil-sized fuel pins and submerged in a pool of deionized water. The fuel elements are ^{235}U enriched uranium/aluminum alloy. The core sits within an annular beryllium reflector while resting on a lower beryllium reflector disc. Semicircular beryllium shims form an upper reflector. As fuel becomes depleted with time, additional shims are added to maintain a constant flux. The fast neutrons which diffuse out of the fuel elements are moderated by the deionized water within the reactor core and the pool. At the center of the fuel cage is a cadmium control rod whose function is to soak up neutrons. Depending on the position of this rod inside the cage relative to the fuel elements, a preset neutron flux can be maintained at a constant level. Concrete slabs, approximately 1 m thick, are placed on top of the pool to act as radiation shields.

Access to inner irradiation sites, located within the beryllium annulus, is via tubes that originate from a remote sample loading station equipped with irradiation controllers to time the duration of irradiation. Samples are usually placed in polyethylene vials, and are pneumatically driven by compressed air to and from the irradiation sites in or near the core.

Activated samples for counting are placed at a predetermined distance from a detector (commonly referred to as the "sample geometry"). Hyperpure germanium (HPGe) solid state detectors, cooled to liquid nitrogen temperature, were used in the work reported here. The detector signals, in the form of charge pulses, are first fed into a pre-amplifier to convert the signals into voltage pulses, then amplified before being fed into a multichannel analyzer. This device converts the analog signals into digital signals; a central processing unit increments the appropriate memory channel corresponding to the particular photon energy. The resulting output, a gamma ray spectrum, is a plot of number of counts as a function of gamma ray energy.

INAA was chosen for the analysis of elements in the Ti horns because of the following attributes: minimal sample handling, multielement capabilities, freedom from possible contamination by dissolution reagents, and non-destructive nature such that sample integrity is maintained.

6.2.3.5 Methods

6.2.3.5.1 Preparation of Blank Solutions for INAA

Solutions in 50-mL Teflon beakers were evaporated to 1 mL or less in the microwave oven. Solutions being evaporated were continuously monitored for spillage or bumping by placing the beakers in a polyethylene container whose inner walls were lined with thin adsorbent paper. In trial runs using water, solution loss was found to be a problem if full microwave power was used at the beginning of evaporation. The most satisfactory program involved initial heating at 20% power for 3 hours, followed by 50% power for 2 hours, and 100% for 1 hour.

Evaporated samples were transferred to 1.5-mL acid-washed polyethylene vials having low backgrounds for the elements of interest. After heat-sealing, each small vial was placed inside a larger 5-mL vial to prevent leakage of the contents during irradiation or transfer. An additional empty 1.5-mL vial was added as a spacer to prevent unwanted movement of the sample vial during transportation between the reactor core and the loading station.

6.2.3.5.2 INAA Procedure for Elemental Analysis of Blank Solutions

Following microwave evaporation, the residues were irradiated at a neutron flux of $1x10^{11}ncm^{-2}sec^{-1}$ in the SLOWPOKE-2 reactor, allowed to decay, and thereafter counted in an HPGe liquid nitrogen cooled detector. The irradiation, decay and counting scheme was 2 minutes each (Table 6.1). The resulting INAA

gamma-ray spectrum was recorded using a 33-MHz 486 IBM computer at a detector to sample distance of 12 cm. Decay corrections and quantitative analysis were performed using an in-house procedure developed by workers in the facility.[222]

6.2.3.5.3 INAA Procedure for Elemental Analysis of Commercial Ti Probe Tip

To determine the elemental composition of the probes used for sonication, a new 0.5-inch diameter tip of titanium alloy, provided by the manufacturer, was irradiated for 2 hours at a neutron flux of $1x10^{12}ncm^{-2}sec^{-1}$. After a decay period of $6x10^{6}$ sec, the tip was counted for $6x10^{4}$ sec at a geometry of 1 cm. The tip was again counted after 400 mg of the total tip mass was removed by etching with 48% HF for 4 minutes.

6.2.3.5.4 Elemental Analysis of Solutions Using ICP-OES

Solutions were aspirated into an ARL 34000 multi-element ICP-OES instrument and determined as before. See section 4.2.4 for details.

6.2.4 RESULTS AND DISCUSSION

6.2.4.1 Extent of Erosion of Ti Probe Tip

A SEM examination of a new 0.75-inch probe tip after several hours of use (Figure 2.4) showed that considerable erosion takes place at the tip, with fragments of metal being released into solution during sonication. Furthermore, the reagents used for ultrasound digestion tend to dissolve the probe material to varying extents during sonication (see section 3.3.7.4). For example, dilute HF

attacks the probe quite vigorously during sonication, whereas attack is slight in the absence of sonication. Over the course of several months, a 19 mm (0.75-inch) diameter probe was reduced to a diameter of 16 mm in the region normally in contact with the reagents. We estimate that 1 to 5 grams of the total probe mass was lost during sonication over this time period. In the next section, results of studies on solution contamination from the probe are presented.

6.2.4.2 INAA Studies of Sonicated Solutions

As a measure of extent of erosion of commercial probes in various solvent mixtures, the amount of Ti released during a 2-hr sonication period was determined by sonicating 80-mL portions of solutions of varying peroxide and sulfuric acid concentrations at full power with a Sonics 0.75" diameter commercial Ti probe. Following microwave evaporation of 80-mL volumes to less than 1 mL for transfer to irradiation vials, activation analysis for short-lived isotopes was performed. See Table 6.1 for acid/peroxide concentrations and for irradiation, cooling and decay times. The results shown in Table 6.1 indicate that the amount of Ti released into solution depends on the reagents employed during sonication. Approximately 11 mg of Ti are released in 2.5% H_2O_2/0.5 M H_2SO_4 solution, compared to only 200 μg in pure water. A 2-hr ultrasound digestion therefore erodes the probe substantially. As mentioned earlier, Ti is of minor importance in most biological systems, so adapting the commercial probe for use in analytical sample dissolution of biological materials without further modifications would not be detrimental. This, however, assumes that the probe is constructed of pure Ti. Gamma-ray spectra of these solutions reveal the presence of other elements as well.

Table 6.1 INAA Results for Ti, Al and V in Sonicated Solutions. Ultrasound treatment was at full power for 2 hrs using a 0.75" diameter commercial Ti alloy Sonics probe; blanks were prepared from the same quantities of reagents, but without sonication. Irradiations were performed at $1x10^{11}$ $ncm^{-2}sec^{-1}$ for 2 min; samples were allowed to decay for 2 min and then counted at a detector-to-sample distance of 12 cm for 2 min. ^{51}Ti(320 keV), ^{28}Al(1779 keV) and ^{52}V(1434 keV) photopeaks were used for quantification. nd means element not detected. Uncertainty $=1\sigma$ based on counting ststistics for a single irradiation.

		amount found (μg).		
solution	treatment	Ti	V	Al
2.5% H_2O_2/0.5 M H_2SO_4	ultrasound	11000±300	522±20	2050±40
2.5% H_2O_2/0.5 M H_2SO_4	blank	nd	nd	nd
0.5% H_2O_2/0.5 M H_2SO_4	ultrasound	3370±90	160±20	730±30
0.5% H_2O_2/0.5 M H_2SO_4	blank	nd	nd	nd
H_2O	ultrasound	190±15	8±2	38±9
H_2O	blank	nd	nd	nd

Vanadium in particular is released in substantial quantities, ranging from 500 μg in 2.5% H_2O_2/0.5 M H_2SO_4 to about 10 μg in pure water. Aluminum was also found, ranging from 2 mg in 2.5% H_2O_2/0.5 M H_2SO_4 to 40 μg in water.

6.2.4.3 ICP-OES Studies of Sonicated Solutions

To further investigate the magnitude of contamination by other elements in the probe, ICP-OES analysis of the sonicated solvents was done. This analytical measurement method was chosen for its excellent detection limits, especially for elements for which INAA is insensitive, such as Pb and Cd. In addition, the INAA determination of elements like Fe, Zn and Cr requires long irradiation times (often 2 to 4 hrs) and long decay times (typically 6 to 21days). With ICP-OES, all of these elements, along with a number of others, can be measured simultaneously in a few minutes.

ICP measurements of solutions sonicated in 80 mL of 0.5% H_2O_2/0.5 M H_2SO_4 were carried out as follows. After sonication as described in the previous section, all solutions were diluted to 100 mL prior to aspiration. To determine the effect of sonication time on the magnitude of contaminants in the solutions, irradiation with a commercial Sonics 0.5" diameter ultrasound probe at full power was performed on individual solutions for 20, 40, 60 and 120 min. The results, presented in Table 6.2, indicate that the greatest concentrations were found for Al and V, suggesting that these elements are likely significant components of the alloy used to manufacture these probes. The concentrations found for these two elements at 120 min are higher than those found in the previous experiment (Table 6.1); this may be due to a higher power density being produced at tip of the 0.5" diameter horn. Detectable quantities of Cu, Cr, Cd, Mn, Sb, Na, Ca, Zn, P, As and Fe were also found. Copper was not detected until after sonication for 40 min, while Sb and Zn were not seen until after sonication for more than an hour.

Table 6.2 ICP-OES Results for 0.5% H_2O_2/0.5 M H_2SO_4 Solutions Sonicated for Varying Times Using a 0.5" Diameter Sonics Probe. Table values are concentrations found, in μg, of elements in 100-mL final volume. nd means element not detected. Uncertainties are ± one standard deviation. Number of replicates: 2, except 4 for 40 min sonication and 1 for Al, Fe, V and P; * means uncertainty is the standard deviation based on 3 measurements of the same solution.

	sonication time (min)				
element	0	20	40	60	120
Mg	nd	nd	nd	nd	nd
Cr	nd	nd	7±1	15±1	32±1
Cu	nd	nd	1.0±0.1	2.4±0.2	3.4±0.2
Cd	nd	nd	nd	nd	0.6±0.3
Sr	nd	nd	nd	nd	nd
Mn	nd	nd	nd	nd	0.20±0.07
Sb	nd	nd	nd	10±3	16±3
As*	157±7	123±3	130±1	125±3	114±5
Fe*	nd	7.3±0.7	21.4±0.2	29.2±0.7	47.1±0.2
V*	nd	170±1	461±3	650±1	1031±4
Pb	nd	nd	nd	nd	nd
Ni	nd	nd	nd	nd	nd
Na	nd	nd	nd	nd	11±3
Ca	nd	nd	nd	nd	3.0±0.1
K	nd	nd	nd	nd	nd
Al*	nd	206±9	578±3	833±2	1362±3
Zn	nd	nd	nd	0.6±0.1	0.7±0.1
P*	3958±60	3227±40	3809±42	3769±35	3929±44

The elements P and As were introduced by contamination from the reagents; the quantities of P and As did not increase with sonication time, indicating that the

probe was not introducing significant amounts of these elements into solution during sonication. This is further illustrated in Figure 6.1, which clearly shows the increase in Al and V, and the constant value for As. Finally, the elements Mg, Sr, Pb, Ni and K were not detected in these solutions after sonication for 2 hr.

6.2.4.4 INAA Studies and HF Etching Experiments on Titanium Probe

Except for P and As, problems with contamination from the reagents used during sonication was found to be negligible, since ICP signals for the blank were found to be no different from those of NANOpure@ water. Further experimentation was done to determine the origin of the observed contamination. Table 6.3 shows the results obtained for a direct INAA analysis of a 2.6684 g solid 0.5-inch diameter tip provided by Sonics. Again Ti, V and Al, quantified via their short lived isotopes in 20-mg portions of the alloy, were found to be major components of the tip; this is consistent with the ICP results shown in Table 6.2. Elements that were quantified via their long-lived radionuclides included: Fe, Zn, Cr, Hf, Ni, Sc, Co, W, Ta, As, Cu and Sb. Gallium and Zr were detected but not quantified. Chromium and Ni were present at levels greater than 150 ppm, while concentrations of the remaining elements were less than 5 ppm. The elements Ba, Mg, Sr and Ca were not detected; this is also consistent with the results shown in Table 6.2.

To determine whether the source of these elements was surface impurities (i.e., possibly introduced during machining) or the alloy itself, approximately 400 mg of the surface of the 0.5-inch diameter tip was removed by etching in 48% HF for 4 minutes.

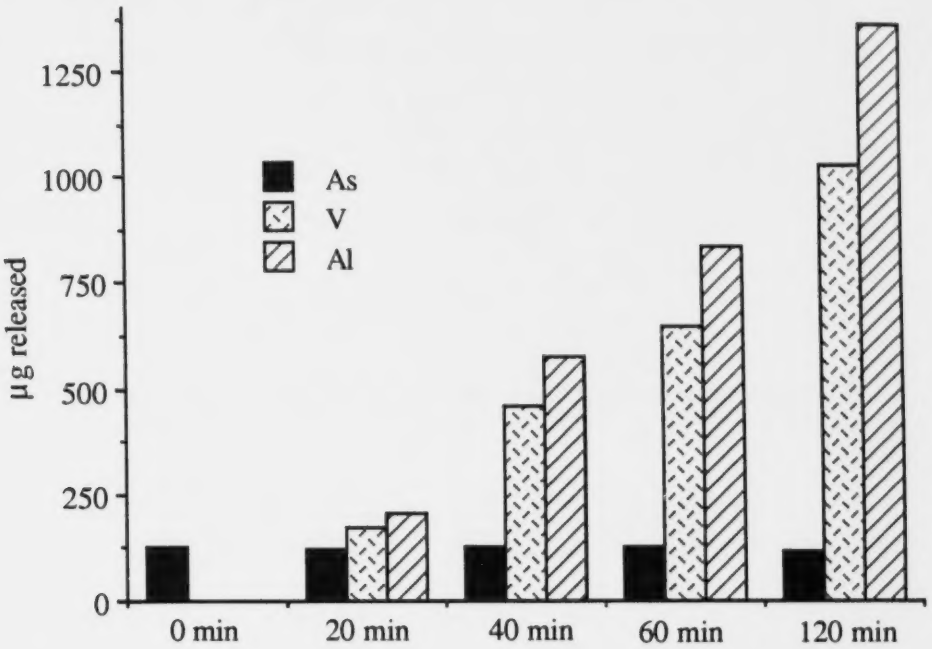


Figure 6.1 Amounts of As, V and Al Appearing in Sonicated Solutions of 0.5% H_2O_2 in 0.5 M H_2SO_4 as a Function of Sonication Time. conditions were: 80 mL of solution, 0.5-inch diameter probe operated at full power.

Table 6.3 INAA Results for a Sonics Commercial Ti Tip. Except where noted otherwise, all values are in ppm; uncertainty = 1σ based on counting statistics for a single sample irradiation.

element	before HF etching	after HF etching
Al (wt. %)	4.2 ± 0.4	not quantified
V (wt. %)	7.1 ± 0.3	not quantified
Mo	46 ± 5	not quantified
Sc	5.5 ± 0.1	5.7 ± 0.1
Ge	not detected	not detected
Fe	0.142 ± 0.003	0.152 ± 0.003
Mg	not detected	not detected
Mn	14±1	not quantified
Sr	not detected	not detected
Ni	142 ± 2	145 ± 3
Hf	0.10±0.02	0.11±0.03
Ba	not detected	not detected
Zn	2.5 ± 0.2	2.5 ± 0.2
Cr	124±8	128±8
Sb	3.7 ± 0.1	3.9 ± 0.1
Ca	not detected	not detected
Co	2.68±0.06	2.74±0.06
W	2.1±0.1	2.0±0.1
Ta	0.074 ± 0.008	0.070 ± 0.009
As	5.6±0.2	5.6±0.2
Cu	2.62 ± 0.06	2.76 ± 0.06

Following repeated washings in deionized water, drying and weighing, the tip was recounted under conditions identical to those described for the pre-etching procedure. The results, also shown in Table 6.3, indicate no statistical differences in the elemental concentrations in the tip before and after the HF etching. Results are shown only for the long-lived isotopes; high specific radioactivity and rapid decay of the short-lived isotopes made it impractical to etch and recount the tip for them.

6.2.5 Summary of Results on Commercial Probe

The results reported here indicate that several elements of biological importance are released from commercial probes during sonication. The quantities vary from element to element, but tend to increase with sonication time at constant solvent composition, and with H_2O_2 concentration at constant sonication time and constant H_2SO_4 concentration. INAA measurements show that the major contaminating elements are distributed within the probe tip material rather than on the surface. The results are consistent with typical values for Ti alloys; for example, the composition of NIST's SRM 173b, Ti base alloy is: Al, 6.36%, V, 4.31%, Mo, 0.013%, and Cu, 0.008%. As a result of these studies, it can be concluded that commercially available Ti probe tips are not suitable for use in ultrasonic dissolution where the resultant solution is to be used for elemental analysis of the above mentioned elements when present in the sample at trace or ultratrace levels. Several alternatives for overcoming this problem were considered. These are explored in the next section.

6.3 MODIFICATION OF HORNS FOR ULTRASOUND DISSOLUTION

6.3.1 Introduction

The results presented in the previous section show that unacceptable levels of impurities are introduced from the sonication probe, resulting in a reduced number of elements that can be reliably measured after digestion using a commercial probe. To avoid this limitation, several options were considered. This section presents both qualitative and quantitative evaluations of the performance of each of these options.

6.3.2 Evaluation of a Teflon Sleeve Cover for Ultrasound Probes

Since Teflon can be obtained in high purity and is resistant to chemical attack, coating the horn with this material should reduce contamination. Use of Teflon also has the potential of allowing HF dissolutions to be carried out for agricultural and geological applications where silicates are present.

6.3.2.1 Experimental

As mentioned in Chapter III, the 0.75" diameter horn has a threaded end to which 0.75" diameter tips can be attached. We purchased, from Ace Glass, a 5" long and 0.75" diameter solid Ti alloy extender rod, manufactured by Sonics, which screws onto the threaded end of the 0.75" diameter horn. The University of Alberta Department of Chemistry Machine Shop shaped the extender to fit tightly into a 3" long, 3 mm thick Teflon sleeve without changing the physical dimensions of the original extender. The sleeve was fastened onto the horn with a pair of screws at the upper end, above solution levels. This design is shown schematically in Figure 6.2.

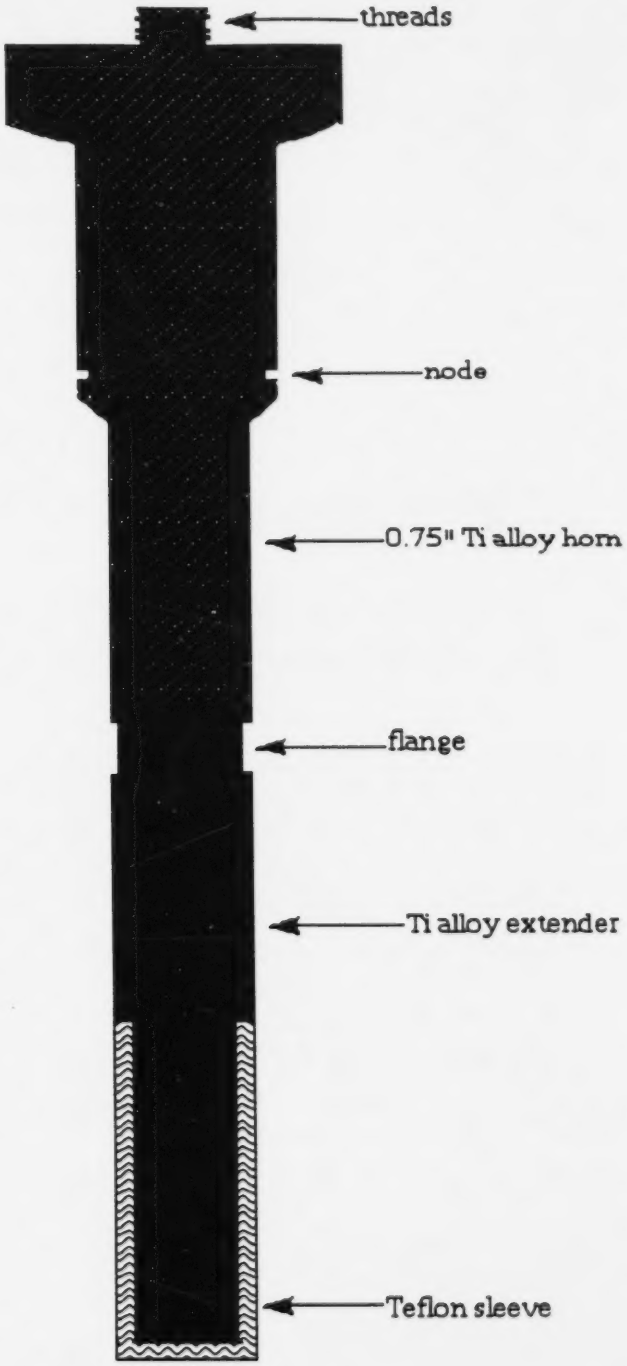


Figure 6.2 Schematic Diagram of a Teflon-Sleeved Commercial Sonics Probe.

6.3.2.2 Results and Discussion

When a new tip or extender is attached to the horn, the ultrasound unit must be tuned prior to use. Tuning involves adjusting the frequency of the power source until resonance of the horn (including extender or tip) is established. The adjustment is made with the horn in air, and must be done quickly to avoid damage to the power supply. Resonance is the condition of maximum vibration, and is a function of the material, shape, mass and length of the horn. The range of tuning provided by the Sonics 9810 processor is only $\pm$ 22 Hz. For this reason, a thin coat of Teflon was used. Under these conditions, the probe tuned without major difficulty.

When the covered probe was used to sonicate water samples, however, there was practically no energy transfer to the solution owing to absorption of the ultrasonic vibrational energy by the layer of Teflon. A condition for the effective transfer of ultrasound energy from the piezoelectric crystal located inside the converter to solutions is that absorption by the system be near zero. The absorption coefficient for Ti is negligible, making it suitable as a universal coupling medium in sonicators.[234] Teflon, on the other hand, is an elastic polymer with a high coefficient of absorption of ultrasound.[235] This absorption resulted in the generation of high temperatures within the Teflon. The problem could have been reduced by use of very thin coatings of Teflon, but erosion damage, as well as increased temperatures, would likely destroy the coating within a few hrs, and so further experimentation on this system was not carried out.

6.3.2.3 Summary

The Teflon sleeve design shown in Figure 6.2 was unsuitable for dissolution of samples with ultrasound because Teflon is not rigid enough to transmit ultrasound energy efficiently to the solution being insonated. Since the use of Ti metal eliminates problems associated with energy losses, the fabrication of horns from pure Ti metal was identified as the best option. The next sections evaluate the performance of three horn designs fabricated from pure Ti metal. The designs studied were pure Ti tips, rods and extenders.

6.3.3 Design and Evaluation of Horn Components Made of Pure Titanium

Several manufacturers of high purity Ti were consulted about obtaining rods of the metal. Several 1-foot long rods of pure Ti, 0.5" in diameter, were obtained from Electronic Space Products International (CA, USA). This silvery metal has the same strength as steel, but is 45% lighter; it is inert to HNO_3, but is attacked by concentrated H_2SO_4, HCl and HF. The purity reported by the manufacturer, was 99.97%, with iron being reported to be the only foreign metal present at 100 ppm. Other elements present in the metal rod, according to the supplier were: carbon, 30 ppm, nitrogen, 80 ppm and oxygen, 100 ppm.[236]

6.3.3.1 Fabrication and Evaluation of Performance of Pure Titanium Tips

With the assumption that the density of the Ti alloy used by Sonics to fabricate their commercial probes (4.20 g/cm^3) was not different enough from that of pure Ti (4.54 g/cm^3) to affect tuning, two pure Ti tips of the same dimensions and shape as the commercial tips (Figure 6.3) were machined, screwed onto a commercial 0.5" diameter probe and tuned. The following section reports on the performance of these tips for ultrasonic dissolutions. All reagents, materials and procedures were as described in section 6.2.

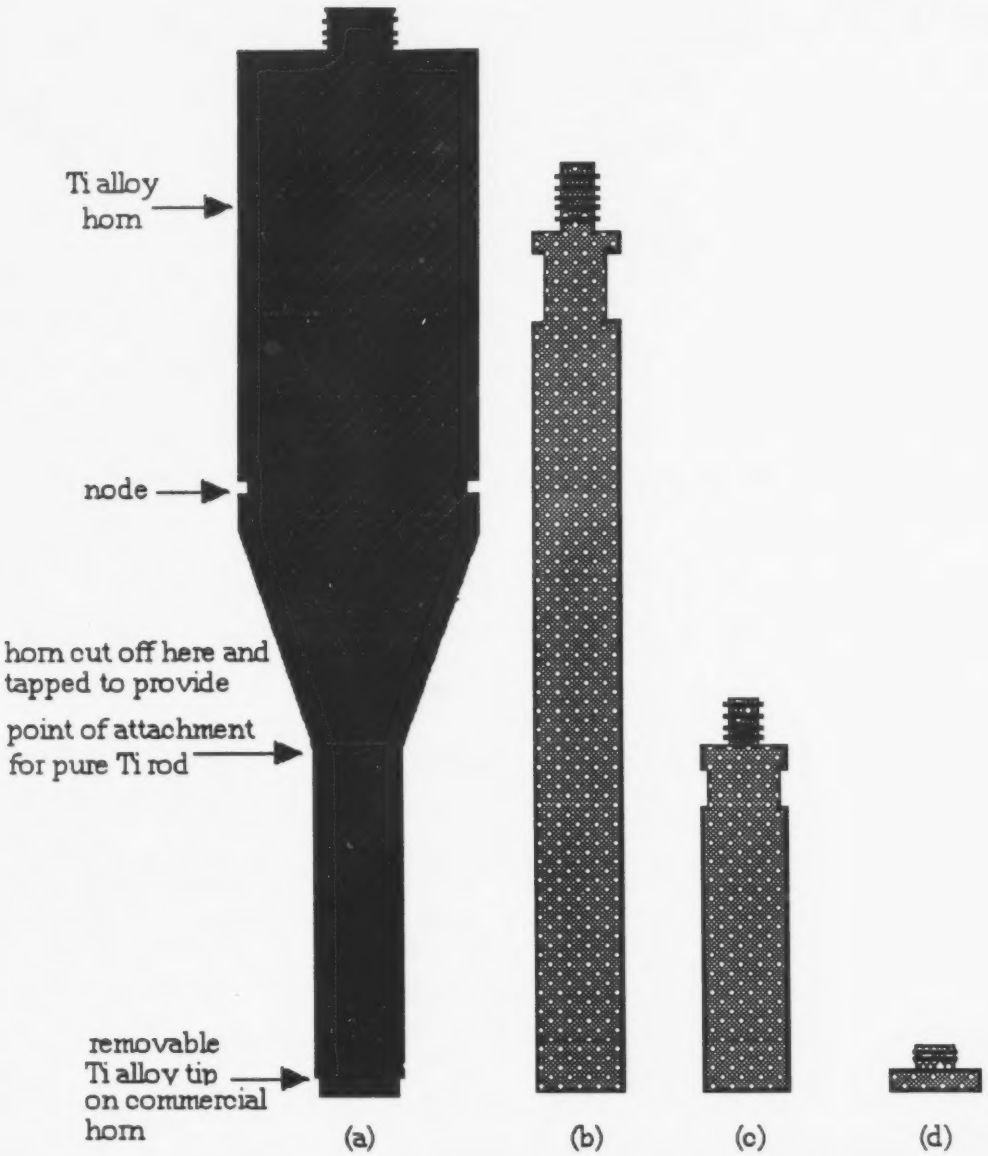


Figure 6.3 Design of Ultrasound Horns for Dissolution. Shown is (a) a Sonics Ti alloy probe with screw-on Ti alloy tip, (b) an extender fabricated from pure Ti, (c) a pure Ti rod fabricated to fit cut-off horn, and (d) a pure Ti tip.

6.3.3.1.1 INAA Study of Trace Elemental Concentrations in High Purity Ti Rods

Compared to commercial Ti alloy samples, the gamma ray spectra of the pure Ti rods were simple. The short irradiation spectra showed a major peak for Ti and three very small peaks indicating the presence of Al, Mn and V. The long irradiation spectra showed the presence of Cr, Ni, Co, Ta, As and Fe. A summary of these results is shown in Table 6.4. When compared to the certificate of analysis supplied by the manufacturer of the Ti metal, the results obtained are satisfactory for all elements except the ones listed. Iron is present in far greater quantities than expected on the basis of the manufacturer's claims; also, the elements Cr, Ni, As and Sb were found present in significant quantities. As a check on the method, irradiation of a similar weight of pure (99.99%) Ti wire was carried out under identical conditions. In the wire, none of these elements were detected. The purity of the probe is clearly less than 99.97%. For sonication of biological materials using probes fabricated from this material, contamination from the elements listed in Table 6.4 is anticipated. This contamination, however, is not a problem for the work described in this thesis because, except for Fe, the other elements are present in the biological materials studied here at concentrations far below the detection capabilities of the technique.

6.3.3.1.2 ICP-OES Analysis of Blanks from Sonication with a Pure Ti Probe Tip

Blank solutions resulting from sonication of 80 mL of 0.5% H_2O_2/0.5 M H_2SO_4 using Ti tips fabricated as described in section 6.3.3.1 above were analyzed by ICP-OES. The results, see Table 6.5, indicate that no detectable contamination was found for Mg, Cd, Sr, Mn, Sb, Ba, Pb, Ni, Na and K even after sonication for 2 hr.

Table 6.4 INAA Results for Pure Ti Metal Used to Fabricate Tips, Rods and Extenders for Ultrasonic Probes. Following washing with acetone, removal of surface layers by etching with HF, rinsing with water and drying at room temperature for 3 days, metal turnings (80 mg) were irradiated at $1x10^{12}$ $ncm^{-2}sec^{-1}$ for 2 hr and counted for 2 hrs after a decay period of 2 weeks except for entries marked with *, where turnings were irradiated for 2 min at $1x10^{11}$ $ncm^{-2}sec^{-1}$ and counted for 3 min at a sample-to-detector distance of 3 cm after a decay period of 10 min. Uncertainties arising from counting statistics are $\pm 1\sigma$.

element	concentration, in μg/g
Al*	82±9
V*	33±3
Mn*	9±2
Cr	129±8
Ni	103±11
As	30±8
Sb	6.5±0.3
Co	0.63±0.06
Ta	0.73±0.09
Fe	410±30

Table 6.5 ICP-OES Results for 0.5% H_2O_2/0.5 M H_2SO_4 Solutions as a Function of Sonication Time Using a 0.5" Diameter Sonics Probe After Replacement of the Ti Alloy Tip with One Fabricated from Pure Ti. Table values are concentrations found, in μg, of elements in 100-mL final volume. nd means element not detected. Number of sonication replicates was 2, except for the 40 min sonication where 4 replicates were run.

	sonication time (min)			
element	20	40	60	120
Mg	nd	nd	nd	nd
Cr	nd	5±1	20±1	25±1
Cu	nd	nd	0.5±0.1	0.6±0.1
Cd	nd	nd	nd	nd
Sr	nd	nd	nd	nd
Mn	nd	nd	nd	nd
Sb	nd	nd	nd	nd
Ba	nd	nd	nd	nd
Fe	5.9±0.4	13.6±0.7	22.6±0.8	25±1
V	98±6	162±7	389±9	438±9
Pb	nd	nd	nd	nd
Ni	nd	nd	nd	nd
Na	nd	nd	nd	nd
Ca	0	0	1.6±0.4	1.5±0.3
As	101±4	133±6	120±2	103±2
Al	117±2	191±3	453±6	519±11
Zn	nd	nd	0.3±0.1	0.4±0.1
P	3457±50	3265±35	3756±40	3689±55

The pure Ti tip therefore eliminates Cd, Mn, Sb and Ca contamination introduced into the blanks by the commercial Ti alloy tip. Manganese, present in the pure Ti tip, did not appear to be leached from the probe during sonication. Even though the pure Ti tip contains far smaller quantities of Al than the commercial probe tip, the blank levels in the sonicated solutions still are high. This may be due in part to solution being insonated not being confined to the length of the pure Ti tip, but rather coming in contact with a significant portion of the Ti alloy of the probe body above the joint with the tip. This could also explain the appearance of small quantities of Cu, Zn, and Ca in the sonicated solutions, whereas these elements are absent in pure Ti.

6.3.3.1.3 Conclusions

Although analyses of blank solutions resulting from sonication using a pure Ti probe tip show reduced levels for many elements, there is still significant contamination from Cu, Cr, Fe, V, Al, Zn, P and As. Except for P and As, contamination of sonicated solutions by the elements listed is thought to arise from three sources, namely, reaction between the reagents and that part of the alloy that comes in contact with the solutions during sonication, the probe itself, and finally the crevice located at the joint where the tip attaches to the horn. The pure Ti rod design described in section 6.3.3.2 below was designed for the purpose of eliminating these problems.

6.3.3.2 Fabrication of Pure Titanium Rods for Ultrasonic Dissolution

6.3.3.2.1 General: The Crevice as a Source of Contamination

A commercial Sonics Ti alloy tip was used in all of the investigations reported in Chapter V. When this tip was replaced by the pure Ti tip described in section 6.3.3.1 above, liquid from previous sonications was observed to be trapped in

the crevice. The tip was carefully removed, then the probe and tip allowed to soak overnight in 50-mL of NANOpure@ water. The resulting solution was aspirated into the ICP by the procedures described in section 4.2.4. Table 6.6 lists the total quantities of all elements detected in the crevice fluid. These results provide confirmation that most P and As contamination in the blanks comes from the reagents. To conclude, the point of attachment of the tip to the probe must be such that it does not come in contact with the material being sonicated. The horn must either be fabricated entirely from pure Ti metal, or pure titanium rods of sufficient length to avoid solution contact must be attached to a commercial horn.

Of these two options, the more viable appeared to be one involving attachment of a rod because of the high cost of pure Ti metal.

6.3.3.2.2 Fabrication of Pure Ti Rods

Several 2" long Ti rods with threaded ends were machined from pure 0.5-inch diameter Ti metal rod. This length was chosen because it allowed the joint to the probe to be located a reasonable distance above the solution level, thereby permitting cell solutions to contact only pure Ti. To keep the length of the probe as close as possible to the commercial design (for tuning), a similar length of metal was cut from the commercial probe. A schematic diagram of a rod fabricated from 0.5" diameter pure Ti metal is shown in Figure 6.3, along with a diagram of the commercial Sonics horn indicating the point of attachment of the pure Ti rod to the commercial probe after it was sawed off and tapped.

Table 6.6 Results of ICP-OES Analyses for Elements Found in Crevice Between Probe and Ti Alloy Tip. Number of replicates=1. Amount found is total in rinse solution.

element	amount found (μg)
Mg	8.1
Cr	8.9
Cu	4.1
Cd	0.88
Sr	0.09
Mn	0.38
Sb	17.3
Ba	0.68
Fe	20.4
V	140
Pb	3.5
Ni	23
Na	175
Ca	15.4
K	167
Al	177
Zn	5.1
P	0
As	0.8

6.3.3.2.3 Tuning of Modified Probe

Attempts to tune the probe modified with the pure Ti rod attached were not successful, nor were attempts to sonicate solutions using this design. The unit was clearly out of range of the limited tuning system of the amplifier. With the assumption that the length or mass of the rod was the source of this problem, approximately 5 mm longer rods were fabricated, and tested for resonance. Successive 0.5 mm sections were cut from the rod and the system tested after each cut to see whether it would resonate, but without success. After this set of experiments it was speculated that the mass of the new probe did not match that of the original closely enough.

The weight of a new commercial probe was compared to that of the probe to which the pure Ti rod had been attached; the commercial probe was found to be 10 g heavier. The density of the alloy was significantly different from that of pure Ti, so much so that replacement of a 2-inch section prevented tuning. The problem was not significant for a 0.25-inch attachment, as can be seen from the Ti tip study reported in section 6.3.3.1. Attempts to keep the length of the probe constant, yet reduce its weight to match that of the commercial probe, also proved unsuccessful.

Engineers at Sonics when contacted about this problem informed us that the point of attachment of the rod was too close to the nodal point of the probe. In ultrasonic engineering terms, the probe can be considered to be composed of two cylinders. The face that attaches to the converter forms one end of a cylinder, 2.7" long and 1.3" in diameter. The face in contact with the sonication medium forms one end of a second cylinder, 2.9" long and 0.5" in diameter. The node occurs at the point where the two cylinders join each other. A plot of stress levels as a function of distance along the length of the probe shows that maximum stress is found at the node. Accommodation of this stress is the reason

why a thick filler of metal is added to this region during manufacture of the commercial horn.[237] Apparently the point of attachment of the pure Ti rod to the cut-off commercial probe was too close to this region of maximum stress to allow adequate tuning.

6.3.3.2.4 Conclusions

Upon moving the joint between a pure Ti tip and the alloy probe away from contact with reagents, the point of attachment of the rod to the commercial probe was found to be too close to the probe node. Tuning of the probe in this configuration was not possible, so sonication could not be done. A possible solution to this problem could be to use an extender to lengthen the probe and move the point of attachment away from the nodal point of the probe. The engineering group at Sonics agreed to machine extender rods from pure Ti provided by us and to tune them to a probe of the same type we are using.

6.3.3.3 Fabrication and Evaluation of Performance of Pure Ti Extenders

A 1-foot long 0.5" diameter pure Ti rod was shipped to Sonics to fabricate and tune several extenders. A typical extender, 4.75" in length, is shown in Figure 6.3(b). One end is threaded, and is designed in such a way that it can screw onto the commercial probe in the same way as a replaceable tip.

6.3.3.3.1 Analysis of Pure Ti Extender Blanks by ICP-OES

As in section 6.3.3.1.2, blank solutions resulting from sonication of 80 mL 0.5% H_2O_2 in 0.5 M H_2SO_4 using a pure Ti extender were analyzed by ICP-OES. Sonication at full power was performed for 20 and 40 min. These times correspond to those reported in Chapter V.

Sonication yielded clear orange/yellow solutions as anticipated. However, a major problem encountered during sonication was the extremely rapid heating rates of the extender at the point of attachment with the probe. Sonication was therefore performed in stages to allow cooling of the probe, i.e., sonication for 10 min followed by cooling for 5 min, and repeating the cycle until full sonication time was achieved.

The results, see Table 6.7, indicate that no detectable contamination was found for Mg, Cu, Cd, Sr, Mn, Ba, Pb, Ni, Na, Ca, Zn and K. There is contamination from P and As, but as shown before, this is likely to be from the reagents. An interesting observation is that contamination from Al and V is negligible, confirming the suspicion that when a pure Ti tip was used in earlier work, contact of solution with the crevice and/or the Ti alloy part of the probe resulted in leaching of impurities.

6.3.3.3.2 Application to Elemental Analysis of Human Hair

To test the reliability and validity of the ultrasound procedure using a pure Ti extender, sonication of four 50-mg portions of human hair in 2.5% H_2O_2/0.5 M H_2SO_4 for 40 min, and quantification of elements present in the digests using the procedure described in section 5.2.3, was performed. For comparison, similar sized test portions of hair were irradiated at a flux of $1x10^{12}$ $ncm^{-2}sec^{-2}$ at the University of Alberta SLOWPOKE-2 nuclear facility and elements found in the hair were quantified via short-lived isotopes. The results obtained by these two independent analytical procedures are shown in Table 6.8. The elements Sr, Cu, Fe, Ca, Zn, Na, Mg and Al were found in the sonicated solutions. When compared to ICP-OES results obtained when a commercial probe was used, see Table 5.11, there is good agreement for Sr, Ca, Zn, Na and Mg. The agreement was not so good for Fe, Cu and Al.

Table 6.7 Results of ICP-OES Analyses for 0.5% H_2O_2/0.5 M H_2SO_4 Solutions Sonicated for Varying Times Using a 0.5" Diameter Sonics Probe in Which the Ti Alloy Tip was Replaced with an Extender Fabricated from Pure Ti. Table values are concentrations found, in μg, of elements in 100-mL final volume. nd means element not detected. Analyses were done in triplicate. Uncertainties are ±1σ.

element	μg/100 mL, after 20 min	μg/100 mL, after 40 min
Mg	nd	nd
Cr	3.2±0.5	10±1
Cu	nd	nd
Cd	nd	nd
Sr	nd	nd
Mn	nd	nd
Sb	3.7±0.2	3.9±0.1
Ba	nd	nd
Fe	31±1	87±3
V	2.2±0.2	3.8±0.3
Pb	nd	nd
Ni	nd	nd
Na	nd	nd
Ca	nd	nd
K	nd	nd
Al	1.8±0.1	1.9±0.1
Zn	nd	nd
P	3356±40	3196±50
As	140±6	138±5

Table 6.8 Results for Analysis of Human Hair. ICP-OES results are for solutions obtained after sonication at full power for 40 min of two 50-mg portions of human hair in 50 mL of 2.5% H_2O_2/0.5 M H_2SO_4 using a 0.5" diameter probe after replacement of the Ti alloy tip with an extender fabricated from pure Ti. Results from Table 5.11 are included for comparison. INAA results were obtained for two 50-mg samples irradiated for 2 min at a neutron flux of $1x10^{12}ncm^{-2}sec^{-1}$, allowed to decay for 60 sec, and then counted for 120 sec at a sample-to-detector distance of 6 cm. Table values are concentrations found, in μg/g. nd means element not detected; nq means not quantified. Uncertainties for INAA results are ±1σ.

	concentration found					
	ICP-OES (Ti alloy tip)		ICP-OES (pure Ti extender)		INAA	
element	sample #1	sample #2	sample #1	sample #2	sample #1	sample #2
Sr	15	17	15	13	nq	nq
Cu	23	15	14	16	15±3	12±3
Mn	nd	nd	nd	nd	0.6±0.1	0.6±0.1
Cd	nd	nd	nd	nd	nq	nq
Fe	40	38	57	47	nq	nq
Pb	nd	nd	nd	nd	nq	nq
I	nq	nq	nq	nq	2.0±0.2	1.1±0.2
Br	nq	nq	nq	nq	8.7±1.0	6.2±1.0
Cl	nq	nq	nq	nq	575±30	560±30
V	nd	nd	nd	nd	nd	nd
Ca	3900	3720	3860	3980	nq	nq
Zn	105	113	111	115	nq	nq
Na	330	250	300	240	nq	nq
Mg	300	420	350	400	nq	nq
Al	1600	800	219	203	206±7	211±8

We conclude that the Fe result cannot be reported with much confidence because of iron contamination in both the pure Ti extender and commercial Ti alloy tip.

The results for Al and Cu in the solutions sonicated with the pure Ti extender show excellent agreement with INAA compared to those obtained with the Ti alloy tip. The improved precision could be due to low blanks arising from low levels of these elements in the pure Ti metal. Similar comparisons for the other elements listed in Table 6.8 cannot be made because only short-lived radionuclides were counted. However, V and Mn levels in the hair samples were below the INAA detection limits.

6.4 SUMMARY OF CONTAMINATION STUDY

When a commercial Sonics ultrasound probe, originally designed for biological cell disruption, is used to dissolve biological materials prior to elemental analysis by ICP-OES, significant elemental contamination is found. Except for As and P, which were shown to come directly from the sulfuric acid used to assist in dissolution, contamination arises from the metal probe itself. Contaminants are uniformly distributed within the probe. Leaching rates vary from element to element, and depend upon solvent composition and duration of sonication.

To eliminate contamination by the probe, several alternatives were explored. Inserting the probe in a tight-fitting sleeve made of Teflon was unsuccessful because of inefficient transfer of ultrasound energy to the solution being insonated. Replacing the commercial Ti alloy tip with tips fabricated from pure Ti was found to eliminate most contaminants, but trapping of solution in the joint between the tip and probe body, and direct contact of solution with the probe body, remained problems. By replacing the lower section of the probe with a

pure Ti rod, both problems are eliminated, but tuning of the probe could not be done. The solution in the end was to replace the tip with an extender rod of pure titanium that had been fabricated and tuned by engineers with the probe manufacturers. With this design, improvements in aluminum results for sonicated human hair samples were realized. No improvement was found for Fe, which was present at significant levels in the pure Ti used in making the probe extender rod.

CHAPTER VII

SUMMARY OF RESULTS AND SUGGESTIONS FOR FURTHER WORK

7.1 Summary

When solid samples are submitted to a laboratory for elemental analyses using solution-based methods such as FAAS and ICP-OES, the analyst is first confronted with the question of how to dissolve them. Most procedures for dissolution of samples prior to measurement involve addition of concentrated acids and application of heat energy to speed the process. This method of dissolving samples, commonly referred to as the "hot-plate" procedure, is time consuming, hazardous, tedious, requires considerable analytical skill and experience, and is prone to loss of elements of interest as well as adventitious contamination from reagents. As techniques for the measurement of elements in a sample have improved in accuracy, precision, speed and efficiency, traditional dissolution procedures have not changed apace, and truly limit the productivity of elemental analysis laboratories of the 1990's.

The research described in this thesis has addressed this problem by use of ultrasound energy to expedite dissolution of biological materials. A commercial Sonics ultrasound probe, originally designed for biological cell disruption, was found suitable as an energy source. The probe employed delivers up to 600 W of 20 kHz ultrasound energy into aqueous suspensions of biological samples in 50 to 100 mL sonication vessels.

Since the rate at which a solid sample is dissolved in a solvent generally depends on the solid/solution interfacial area, a first step is often the formation of a heterogeneous two-phase solid-liquid system. Accordingly, the first part of this work involved a study of the fundamental physical processes that occur at the

surface of sample particles suspended in a solvent. Photomicrographs of surfaces of sonicated chalk samples showed that when ultrasound energy is passed through water, it is concentrated and amplified by cavitation. Acoustic cavitation near the surface of aluminum foil resulted in pitting, erosion and fragmentation of the foil. Acoustic streaming was seen to propel fragments of metal at high velocities, resulting in many interparticle collisions. Scanning electron microscopic studies of titanium metal surfaces showed that cavitation could be so powerful that metal fragments are ripped from the surface during sonication. Iodimetric analysis of the residue remaining after sonication of copper metal for an hour revealed that approximately 20 mg of copper was removed from the surface. Application of ultrasound to hemin, powdered liver and cellulose showed that cavitation increased the solvent-accessible surface area by several orders of magnitude, depending on the length of sonication and the material under investigation. This *sonomechanical* effect, which is absent when heat energy is used, enhances rates of dissolution because reactions with the solvent occur at the surface of the solid.

Thermodynamic calculations show that the concentration of ultrasound energy by cavitation, and the subsequent amplification of the pressure waves when passed into water, lead to high localized temperatures (5000 K) and pressures (1000 atm). These conditions lead to the generation of substantial amounts of oxidants from water in equilibrium with atmospheric gases. This *sonochemical* effect, also absent when conventional heating is used, leads to enhanced dissolution rates of samples with little or no external reagent addition. Since oxidants did not form to any significant extent in water deaerated by boiling prior to sonication, it was concluded that dissolved gases must be present for efficient cavitation.

This cavitation effect was investigated further to find optimum conditions for the production of oxidizing species from water during sonication. Of the common laboratory gases studied, nitrogen was found to be a poor cavitation gas, while argon as a cavitation gas gave the highest concentration of oxidants. Anomalous behavior was observed when oxygen was used, in that oxidant levels were found to be very high at the onset of sonication, but decreased soon after. The high initial levels could not be maintained because of ultrasound degassing and a rapid increase in the solution temperature. Attempts to counter degassing by bubbling oxygen continuously during sonication were unsuccessful, as were attempts to thermostat the solution.

Next the effect of acid in the production of oxidants in sonicated water samples was investigated. In dilute H_2SO_4, oxidant levels increase with acid concentration; the maximum yield was obtained at 0.5 M. Solutions more concentrated than 0.5 M gave lower yields, owing to the high H^+ concentration, and not to high sulfate concentrations. In dilute HNO_3 and HCl the yield of oxidants was found to decrease with increasing concentration. This indirectly suggests that cavitation leads primarily to the production of free radicals from water, although the exact identity of the free radical species was not studied. It is suggested that at higher concentrations of HNO_3 or HCl, nitrogen- or chlorine-containing radical scavengers reduce the yield of oxidants produced by sonication of water.

Finally, sonication in dilute HF led to a violent reaction with the probe, a behavior similar to that observed when Ti metal is immersed in concentrated (25.7 M) HF. Clearly ultrasound enhances chemical reactivity, either by physical or chemical processes, or both. Sonication of mixtures of HF and HNO_3 generate copious vapors of brown nitrogen dioxide. Overall, optimum conditions for

producing oxidants were found to be sonication in 0.5 M H_2SO_4 while bubbling a steady stream of oxygen gas through the solution.

To compare sonication of solid samples with dissolution by heating on a hot plate, several biological materials were investigated. Sonication of $Ni(DMG)_2$ in H_2O resulted in the release of most of the Ni from the complex, although the release was not quantitative. Heating to boiling in water, on the other hand, had little effect. Sonication in 0.5 M H_2SO_4 with oxygen bubbling led to complete dissolution within 3 minutes. Dissolution, however, was found to be due to protonation of the ligand. Sonication of 100-mg portions of bovine hemin in several acid mixtures did not produce 100% dissolution; nitric acid was the most effective. To achieve 100% dissolution of hemin, it was necessary to boost the oxidizing capacity of sonicated solutions by addition of H_2O_2. Since oxidation by H_2O_2 shows a strong pH dependence, it was found advantageous to add acid. Thus, sonication of hemin in 2.5% H_2O_2/0.5 M H_2SO_4 as solvent resulted in complete dissolution with no traces of residue in less than 20 minutes. Dissolution by heating in various acids, with or without H_2O_2, on the other hand, was found to be very slow. Use of H_2SO_4 as acid is important; complete dissolution of hemin by sonication in 2.5% H_2O_2 with 1.0 M HNO_3 or HCl was not observed, even at long times.

Sonication of several biological reference materials from NIST (USA), NRC (Canada) and NIES (Japan) in 2.5% H_2O_2/0.5 M H_2SO_4 gave results in good agreement with certified values for most elements. Samples containing fat, such as whole milk powder were difficult to dissolve completely. For whole milk powder, experimental results did not match certified values for any element except zinc.

In conclusion, the ultrasound method of dissolving samples proposed here shows promise for application to a wide range of biological materials. It is faster

in general than traditional methods based on heating samples on a hot plate. With proper control of sample weight and H_2O_2 concentration, dissolution rates by ultrasound approach those attainable by current microwave acid digestion procedures. A particular advantage of the ultrasound method is that smaller reagent quantities may be used because oxidants are produced *in situ*. This can make routine analyses less costly, and reduces the likelihood of contamination.

The greatest disadvantage to using the commercial probe provided with the apparatus employed in this work for analytical dissolution is contamination by elements in the alloy used to construct the probe. Instrumental neutron activation analysis (INAA) and ICP-OES showed that Al, V, Fe, Cu and Zn leached into solution from the probe during sonication, resulting in high backgrounds and poor precision for these elements. Enclosure of the probe in a sleeve fabricated from Teflon resulted in reduced sonic output to the solution and excessive energy absorption and heating of the Teflon. Replacement of commercial Ti alloy tips with ones fabricated from pure Ti reduced contamination, but did not eliminate it completely because the sample solutions still came in contact with the probe above the tip. Attachment of 2" long pure Ti rods to the commercial probe resulted in problems with tuning of the probe. The best results were obtained when a 4.75" long extender was fabricated, attached to a commercial probe, and the unit tuned by the manufacturer.

When applied to dissolution of human hair, good accuracy and precision for those elements detectable by ICP-OES was found when compared to results obtained by INAA. The extender design, however, has its flaws. First, excessive heating at the probe-extender interface required sonication to be done in stages. The unit had to be manually switched off for 5 minutes following sonication for 10 minutes in order to cool the probe. Secondly, contamination from Fe was worse than when a commercial Ti probe was used. This could probably be

eliminated by use of extenders fabricated from Ti metal with purity greater than 99.97%. This would be costly. On the other hand, iron was the only metal impurity found in the 99.97% titanium at levels sufficient to cause concern.

To summarize, it has been demonstrated that commercial ultrasound probes can be used to dissolve quickly a wide range of solid biological samples without the need of adding large quantities of concentrated and sometimes hazardous acids. The equipment needed to achieve ultrasound dissolution is simple, easy to operate and commercially available at reasonable cost. This research forms the starting point for the application of sonochemistry, a field that has been an exclusive domain for synthetic chemists since the first sonochemically assisted synthesis was reported in 1978,[145] to analytical sample preparation.

7.2 Suggestions for Further Work

Even though this study has shown that ultrasound provides a potentially powerful new mechanism for the dissolution of solid samples, further research and development of this technology would be a worthwhile investment to the advancement of instrumental analytical chemistry. Below is an outline of suggestions for future research in this area.

7.2.1 Identification of Chemical Species Produced by Cavitation

In chapter III, the species formed from water during acoustic cavitation were not specifically identified. They were broadly classified as oxidants that liberate I_2 from KI solutions. They could be any radical such as OH, HO_2, etc.[198] or other species which might react directly with I^- to form I_2. Identification of these species could help establish optimum conditions for sample decomposition.

It is also possible that cavitation may cleave hydrogen peroxide to form hydroxyl radicals in the same way that uv photolysis does in enhanced

oxidation processes.[238] Since hydroxyl radicals are more reactive than hydrogen peroxide itself, it is possible that this could be the reason why ultrasound dissolution of organic compounds is faster than heating. ESR studies might be used to obtain information on this process.

During ultrasound dissolution of biological materials in H_2O_2, it was observed that copious amounts of gases were evolved. A variety of gases might be produced. It would be worth identifying these, using techniques such as GC. It may also help to know the products of ultrasound decomposition in various solvents. This is important because of possible incomplete decomposition, even though samples are clear in appearance.[239] For this purpose, techniques such as HPLC and ion chromatography could be employed. On the other hand, because of the high temperatures that prevail in an inductively coupled plasma, this information may not be critical.

7.2.2 Application to Materials Other Than Biological Samples

Applications to non-biological materials such as those of geological and metallurgical origin could be investigated. Organic fractions in soils could be decomposed by the procedure developed for biological samples; thereafter, siliceous material could be removed with HF in a microwave oven. Thus these two methods could complement each other. Another way in which this method might be applied to siliceous samples is by revisiting the Teflon sleeve design proposed in chapter VI. If this design could be implemented, HF digestions using ultrasound would be possible.

Lastly, basic, rather than acidic, microwave digestion procedures for refractory metal oxides have recently been reported.[240] Studies of ultrasound digestion in basic media is another area that can be explored.

7.2.3 Kinetic Studies

Since the rate of dissolution of organic compounds by use of ultrasound is controlled by both physical and chemical factors, it may be possible to formulate rate laws for this process. The simplest approach would be to assume that the overall rate law is a sum of two terms, namely surface area and reagent concentration. BET surface area measurements could be used to determine the surface area dependence, whereas conventional techniques such as spectroscopy could be used to determine the concentration dependence.

7.2.4 Ultrasound Equipment

Like most commercial units, the Sonics probe used in these studies resonates at a fixed 20 kHz, thus limiting studies to this frequency. There is growing evidence that, contrary to popular belief,[220] frequency does affect chemical reactions. Therefore research into technology for producing probes that have multi-frequency operation is needed to determine the effects of frequency on dissolution rates and conditions.

It was shown in this study that the rate of production of oxidant species depends on ultrasound power. Quantities of oxidants produced during sonication of water at full power for one hour were in the μM range, a concentration not sufficient to dissolve 100-mg portions of sample without external addition of oxidants such as H_2O_2. The development of more powerful probes would produce higher levels of oxidants; these levels might be sufficient to achieve complete decomposition without addition of external reagents.

It may also be necessary to redesign the sonication vessels to fit differing analytical applications. First, there is a need to ensure that all the sample is under the cavitational plume at all times. Therefore the spacial distribution of ultrasound from source through the sample suspension to the bottom of the

sonication vessel needs to be considered. Secondly, to ensure reasonable lifetimes for the apparatus and to avoid expensive replacement of the converter, the sonication vessel needs to be designed to prevent fumes evolved during digestion from reaching the electronic components.

An obvious limitation of this method is the relatively long dissolution time of one sample per hour. If more powerful processors can be developed, multiprobe systems might be feasible that could process several samples at a time.[241]

Finally, computer-controlled digestions using ultrasound could be considered. This would enable operation of the unit in a "hands-off" mode. One advantage of such systems could be a reduction in costs associated with labor. Another is the use of robotics so that samples can be digested by day or night, in attended or unattended operation. To avoid manual control of power output, sonication and cooling cycles, the pulser inside the ultrasound processor could be replaced by a computer which, after development of dissolution schemes for each application, could routinely do this task. Perhaps ultrasound could also be adapted to the ultimate goal of true automation whereby computers control all steps in an analysis, from sample preparation to data analysis and report generation, with in-built feedback mechanisms (artificial intelligence).

References

1. R. F. Burk, "Trace Elements in Human Health and Disease", vol.2, A.S. Prasad and D. Oberleas (eds), Academic Press, New York, (1976), 105.

2. K. Okamoto, *Res. Rep. Natl. Inst. Environ. Stud.*, **18** (1980), 5.

3. J. Tariq, M. Jaffar and M. Ashraf, *Fish Res.*, **19** (1994), 131.

4. R. Pal, R. Nath and K. D. Gill, *Pharmacology and Toxicology*, **73** (1993), 209.

5. D. Purves, "Trace Element Contamination of the Environment", Elsevier Scientific Publishing Co., New York, 1976.

6. L. Ember, *Chemical and Engineering News*, October 25, 1993, 7.

7. S. B. Markowitz, S. Klitzman, C. M. Nunez, A. A. Munshi, W. S. Kim, J. Eisinger and P. J. Landrigan, *J. Am. Med. Assoc.*, **271** (1994), 932.

8. L. S. Maresky and S. R. Grobler, *Sci. Total Environ.*, **136** (1993), 43.

9. B. G. Kendler and D. J. Pirone, *Am. Biol. Teach.*, **56** (1994), 152.

10. P. Rasmussen, "Pb in roadside flora and fauna in Swaziland", A Research Report of the Department of Chemistry, University of Swaziland. 1985.

11. L. T. Ou, J. E. Thomas and W. Jing, *Bull. Environ. Contam. Toxicol.*, **52** (1994), 238.

12. A. D. Moehr, D. W. Roberts, P. E. Phillips and R. G. Evans, *J. Environ. Health*, **56** (1993), 20.

13. S. Gozzo, G. Perretta, V. Monaco, U. Andreozzi and E. Rosiello, *Ecotoxicol. Environ. Saf.*, **26** (1993), 293.

14. E. Andrassi, S. Monika, I. Saray, L. Bezur, L. Ernyei, and A. Reffy, *Sci. Total Environ.*, **139** (1993), 399.

15. T. Suzuki, T. Hongo, J. Yoshinaga, H Imai, M. Nakazawa, N. Matuo and H. Akagi, *Arch. Environ. Health*, **48** (1993), 221.

16. D. B. Milne and G. M. Schelkoph, *Anal. Chem.*, **60** (1988), 2060.

17. M. H. Wong and S. T. Chiu, *Environ. Tech.*, **14** (1993), 1155.

18. M. T. Arcos, M. C. Ancin, J. C. Echeverria, A. Gonzales and J. J. Garrido, *J. Agr. Food Chem.*, **41** (1993), 2333.

19. L. Jorhem and B. Sundstrom, *J. Food Comp. Anal.*, **6** (1993), 223.

20. G. Vivekanandan, V. Krishnasamy, K. Ayyadurai and C. S. Swaminathan, *J. Environ Biol.*, **14** (1993), 261.

21. R. Zeisler, P. Ostapczuk, S. F. Stone and M. Steoppler, *Sci. Total. Environ.*, **139** (1993), 403.

22. B. Kratochvil, National Bureau of Standards Research Report, NBS, Gaithersburg, USA. 1983.

23. O. M. Poulsen, J. M. Christensen, E. Sabbioni and M. T. Van der Venne, *Sci. Total Environ.*, **141** (1994), 197.

24. L. A. Jorgensen and B. Pedersen, *Marine Pollution Bulletin*, **28** (1994), 24.

25. M. Ferreti, R. Udisti and E. Baebolani, *Fres. J. Anal. Chem.*, **347** (1993), 467.

26. W. Worthy, R. Dagani, S. Stinson and J. Krieger, *Chemical and Engineering News*, March 24, 1986, 21.

27. U. von Buchstab, *Canadian Research*, **15** (1982), 33.

28. H. Agemain, D. P. Sturtevant and K. D. Austen, *Analyst*, **105** (1980), 125.

29. M. Verlinden, *Talanta*, **29** (1982), 875.

30. G. Horlick, *Phil. Trans. R. Soc. Lond. A*, **305** (1982), 681.

31. J. Grasselli, *Anal. Chem.*, **64** (1992), 677A.

32. G. Horlick, *Chemistry in Canada*, May 1983, 21.

33. R. E. Clement, C. J. Koester and G. Eiceman, *Anal. Chem.*, **65** (1993), 85R.

34. C. A. Kirchner, G. A. Eagle and H. F. K. O. Hennig, *Intern. J. Environ. Anal. Chem.*, **32** (1988), 9.

35. K. Jackson and P. Newman, *Analyst*, **108** (1983), 261.

36. M. Berrow and M. Stein, *Analyst*, **108** (1983), 277.

37. J. Cansul, T. Fries and P. Lamonthe, *Anal. Chem.*, **58** (1986), 1881.

38. T. T. Gorsuch. "The Destruction of Organic Matter", Pergamon, New York, 1970.

39. D. C Bogen in: "Treatise in Analytical Chemistry", P. J. Elving, E. Grushka and I. M. Kolthoff, Eds., Part 1, "Theory and Practice," 2nd ed., Vol. 5, John Wiley and Sons, N.Y., 1982.

40. E. C Dunlop and C. R. Ginnard in: "Treatise in Analytical Chemistry," P. J. Elving, E. Grushka and I. M. Kolthoff, Eds., Part 1, "Theory and Practice," 2nd ed., Vol. 5, John Wiley and Sons, N.Y., 1982.

41. T. S. Ma, C. Y. Wang, and M. Gutterson, *Anal. Chem.*, **54** (1982), 87R.

42. R. Bock, "A Handbook of Decomposition Methods in Analytical Chemistry", International Textbook Co., London, 1979.

43. T. T. Gorsuch, *Analyst*, **87** (1962), 112.

44. T. T. Gorsuch, *Analyst*, **84** (1959), 135.

45. P. Strohal, S. Lulic, and O. Jelasavcic, *Analyst*, **94** (1969), 678.

46. C. A. Rowan, O. T. Zajicek, and E. J. Calabreses, *Anal. Chem.*, **54** (1982), 149.

47. R. L. Dahlquist and J. W. Knoll, *Appl. Spectrosc.*, **32** (1978), 1.

48. J. L. M. DeBoer and F. J. M. J. Maessen, *Spectrochim. Acta.*, **38B** (1983), 739.

49. N. A. Arafat and W. A. Glooschenko, *Analyst*, **106** (1981),1174.

50. M. Verlinden, *Talanta*, **29** (1982), 875.

51. T. E. Green and M. Turley, in: "Treatise on Analytical Chemistry", I. M Kolthoff and P. J. Elving (eds), Part II, vol.7, p139. Inter science, New York, 1961.

52. W. H. Evans, J. I. Read and B. E. Lucas, *Analyst*, **103** (1978), 580.

53. "Properties and Essential Information for the safe Handling and Use of Perchloric Acid Solutions", Chemical Safety Data Sheet SD-11, Manufacturing Chemists Association, Washington, D.C., 1965.

54. A. Schilt, "Perchloric Acid and Perchlorates", G. F. Smith Chemical Company, Ohio, 1979.

55. S. Mamba and B. Kratochvil, *Can. J. Chem.*, **68** (1989), 360.

56. C. A. Johnson, J. F. Lewin and P. A. Fleming, *Anal. Chim. Acta.*, **82** (1976), 79.

57. R. J. Hall and P. L. Gupta, *Analyst*, **94** (1969), 292.

58. A. Milne, *American Laboratory*, November 1990, p40D.

59. M. S. Vigler, A. W. Varnes and H. A.. Strecker, *American Laboratory*, **12** (1980), 31.

60. T. S. Ma and V. Horak, "Microscale Manipulations in Chemistry", John Wiley and Sons, New York, 1976.

61. M. R. Hendzel and D. M. Jamieson, *Anal. Chem.*, **48** (1976), 926.

62. G. Knapp, *Fresenius Z. Anal. Chem.*, **274** (1975), 271.

63. H. Agemain, D. P. Sturtevant and K. D. Austen, *Analyst*, **105** (1980), 125.

64. G. Kaiser, D. Kotz, G. Tolg, G. Knapp, B. Maichin, and H. Spitzy, *Fresenius Z. Anal. Chem.*, **291** (1978), 278.

65. J. Helfrich, Zymark Corporation Technical Document, **1**(1990), 2.

66. J. Helfrich, *Advances in Laboratory Automation Robotics*, **5** (1990), 221.

67. R. Uhrberg, *Anal. Chem.*, **54** (1906), 1882.

68. A. Van Eenbergen and E. Bruninx, *Anal. Chim. Acta.*, **98** (1978), 405.

69. L. Kotz, G. Henze, G. Kaiser, S. Pahlke, M. Veber and G. Tolg, *Talanta*, **26** (1979), 681.

70. P. Anon, "Parr Acid Digeston Bombs" (Parr Instrument Co., Moline, IL, 1976) Bulletin 4745.

71. F. McGuiness, Delta Scientific Laboratory Products Ltd.(1990), Missisagua, Ontario.

72. H. Matusiewicz, *Chem. Anal.(Warsaw)*, **28** (1983), 439.

73. E. Jackwerth and S. Gomiscek, *Pure Appl. Chem.*, **56** (1984), 479.

74. H. Matusiewicz and R. M. Barnes, *Anal. Chem.*, **57** (1985), 406.

75. S. A. Katz, S. W. Jennis, T. Mount, R. E. Tout and A. Chatt, *Intern. J. Environ. Anal. Chem.*, **9** (1981), 209.

76. B. Bernas, *Anal.Chem.*, **40** (1968), 1682.

77. F. J. Langmyhr and P. E. Paus, *Anal. Chim. Acta.*, **43** (1968), 397.

78. A. Abu-Samra, S. Koirtyohann and J. Morris, *Anal. Chem.*, **47** (1975), 1475.

79. Y. Kalra, D. Maynard and F. Radford, *Can. J. For. Res.*, **19** (1989), 981.

80. S. Mamba, "Microwave Acid Dissolution of Swaziland Soils" M.Sc. thesis, 1988. University of Alberta, Edmonton, Alberta Canada.

81. M. Robertson, *Canadian Laboratory*, **2**(4), September 1990, 18.

82. R. T. White and G. E. Douthit, Jr., *J. Assoc. Off. Anal. Chem.*, **68** (1985), 766.

83. C. J. Warren, B. Xing and M. J. Dudas, *Can. J. Soil Sci.*, **70** (1990), 617.

84. C. G. Millward and P. D. Kluckner, *J. Anal. At. Spectrom.*,**4** (1989), 709.

85. A. Morales, F Pomares, M. de la Guardia and A. Salvador, *J. Anal. At. Spectrom.*, **4** (1989), 329.

86. H. Matusiewicz, R.Sturgeon and S.S.Berman, *J. Anal. At. Spectrom.*, **4** (1989), 323.

87. B. McCarthy, *J. Am. Soc. Brew. Chem.*, **47** (1989), 91.

88. M. A. Navarro, M. C. Lopez and M. V. Sanchez, *J. Agr. Food Chem.*, **39** (1991), 2223.

89. G. Schelkoph and D. Milne, *Anal. Chem.*, **60** (1988), 2060.

90. B. D. Zehr, J. P. Van Kuren and H. M. McMahon, *Anal. Chem.*, **66** (1994), 2194.

91. B. D. Zehr and M. A. Fedorchak, *Am. Lab.*, March 1991, 40.

92. B. D. Zehr, *Am. Lab.*, September 1992, 24.

93. B. D. Zehr and T. J. Zehr, *Spectroscopy*, **6** (1991), 44.

94. A. Grillo and M. Moses, *Am. Lab.*, September 1992, 58.

95. Method 3015, Revision 0, Nov. 1990. "Microwave Assisted Acid Digestion of Aqueous Samples and Extracts; The EPA Resource Conservation and Recovery Act (RCRA)", program SW846 (1990).

96. Method 3051, Revision 0, Nov. 1990. "Microwave Assisted Acid Digestion of Sediments, Sludges, soils and Oils; The EPA Resource Conservation and Recovery Act (RCRA)", program SW846 (1990).

97. R. D. Smith, "Microwave Power in Industries", Report ERPI-EM 3645, 1984. Electrical Power Research Institute, Palo Alto, Calif., USA.

98. K. E. Haque, *Energy, Mines and Resources Canada*, 1984, 327.

99. J. W. Walkiewicz, G. Kazonich and S. L. McGill, *Minerals and Mineral Processing*, February 1988, 39.

100. T. T. Chen, J. E. Dutrizac, K. E. Haque, W. Wyslouzil and S. Kashyap, *Canadian Metallurgical Quarterly*, **23** (1984), 349.

101. D. R. Baghurst and D. M. P. Mingos, *J. Chem. Soc., Chem. Commun.*, 1988, 829.

102. A. Grillo, *Spectrosc.*, **5** (1990), 55.

103. M. E.Tatro, *Environ. Lab.*, Feb/Mar 1991, 28.

104. G. Legere and E. D. Salin, paper presented at 76-th Canadian Society for Chemistry conference, 30 May - 3 June 1993, Sherbrooke, Quebec, Canada.

105. J. Helfrich, Zymark Corporation Technical Document, **1** (1990), 2.

106. V. Karanassois, F. H. Liu and E. D. Salin, *J. Anal. At. Spectrom.*, **6** (1991), 457.

107. G. Horlick, *Anal. Chem.*, **54** (1982), 276R.

108. N. Miller-Ihli, *J. Anal. At. Spectrom.*, **4** (1989), 295.

109. K. W. Jackson and A. P. Newman, *Analyst*, **108** (1983), 261.

110. M. J. Dudas, G. A. Spiers and L. W. Hodgins, *Clays and Clay Minerals*, **31** (1983), 397.

111. J. G. Williams, A. Grey, P. Norman and L. Ebdon, *J. Anal. At. Spectrom.*, **2** (1987), 469.

112. C. Cabrera, M. L. Lorenzo, C. Gallego, M. C. Lopez and E. Lillo, *J. Agric. Food Chem.*, **42** (1994), 126.

113. R. F. Browner and A. W. Boorn, *Anal. Chem.*, **56** (1984), 786A.

114. R. M. Barnes, "Emission Spectroscopy", Chapter 1. Dowden, Hutchinson and Ross, Inc., Stroudsburg, Penn., 1976.

115. E. D. Salin, G. Horlick, *Anal. Chem.*, **51** (1979), 2089.

116. V. Majidi and J. A. Holcombe, *Spectrochim. Acta.*, **45B** (1990), 753.

117. V. Majidi and J. A. Holcombe, *J. Anal. At. Spectrom.*, **4** (1989), 423.

118. Y. Shao and G. Horlick, *Appl. Spectrosc.*, **40** (1986), p386.

119. D. Sommer and K. Ohls, *Fresenius Z. Anal Chem.*, **304** (1980), 97.

120. T. J. Mason and J. P. Lorimer, "Sonochemistry: Theory, Aplications and uses of Ultrasound in Chemistry", page 3. John Willey and Sons, New York, 1988.

121. G. J. Price, "Current Trends in Sonochemistry", page 1. Royal Society of Chemistry, Cambridge, 1992.

122. F. Galton, "Inquiries into Human Faculty and Development", Macmillan, 1883.

123. J. Curie and P. Curie, *Compt. Rend.*, **91** (1880), 294.

124. J. Curie and P. Curie, *Compt. Rend.*, **93** (1881), 1137.

125. N. K. Rossing and C. Quanren, *Ultrasonics*, **23** (1985), 173.

126. J. C. Birnholtz and E. E. Farrell, *American Scientist*, **72** (1984), 608.

127. A. M. Al Karmi, M. A. Dinno, D. A. Stoltz, L. A. Crum and J. C. Mathews, *Ultrasound in Medicine and Biology*, **20** (1994), 73.

128. C. Damianou and K. Hynynen, *Ultrasound in Medine and Biology*, **19** (1993), 777.

129. J. Webster and B. A. Stone, *Aquatic Botany*, **47** (1994), 29.

130. M. H. Selima, A. Abd El Baky, M. K. Zahra and H. M. Hassan, *Egyptian Journal of Microbiology*, **25** (1993), 401.

131. M. A. Messman and B. P. Weiss, *J. Agric. Food Chem.*, **41** (1993), 1085.

132. B. V. R. Sastry, S. R. Kambam, G. Singh and J. J. Franks, *Journal of Ocular Pharmacology*, **10** (1994), 195.

133. E. Andreasson and P. A. Albertson, *Biochim. Biophys. Acta.*, **1141** (1993), 175.

134. P. Knight, The Financial Post, February 8, 1991, page 12.

135. Y. Nakahara, K. Takahashi and K. Konuma, *Forensic Science International*, **63** (1993), 109

136. R. M. Schrock, J. W. Beeman, D. W. Rondorf and P. V. Haner, *Trans. Am. Fish Soc.*, **123** (1994), 223.

137. M. Sandberg, X. Li, S. Folestad, S. G. Webber and O. Orwar, *Anal. Biochem.*, **217** (1994), 48.

138. M. T. Downes, L. Harstich and W. F. Vincent, *J. Appl. Phyc.*, **5** (1993), 623.

139. C. A. Crouse, J. D. Ban and J. K. D'Alessio, *Biotechniques*, **15** (1993), 644.

140. G. J. Price, "Current Trends in Sonochemistry", page 176. Royal Society of Chemistry, Cambridge, 1992.

141. W. T. Richards and A. L. Loomis, *J. Am. Chem. Soc.*, **49** (1927), 3086.

142. P. Riesz, D. Berdahl and C. L. Christman, *Environ. Health Persp.*, **64** (1985), 233.

143. P. Renaud, *Bull. Soc. Chim. Fr.*, 1950, 1044.

144. R. A. Abdulla, *Aldrichimica Acta*, **21**(1988), 31.

145. A. J. Fry, G. S. Ginsburg and R. A. Parente, *J. Che. Soc. Chem. Commun.*, 1978, 1040.

146. S. Moon, L. Duchin and J. V. Cooney, *Tetrahedron Lett.*, **20** (1979), 3917.

147. T. J. Mason, J. P. Lorimer and B. P. Mistry, *Tetrahedron Lett.*, **23** (1982), 5363.

148. J. C. Menendez, G. G. Trigo and M. M. Sollhuber, *Tetrahedron Lett.*, **27** (1986), 3285.

149. J. Lindley, J. P. Lorimer and T. J. Mason, *Ultrasonics*, **24** (1986), 293.

150. J. L. Luche and J. C. Damiano, *J. Am. Chem. Soc.*, **102** (1980), 7926.

151. P. Boudjouk and B. H. Han, *Tetrahedron Lett.*, **22** (1981), 3813.

152. J. Yamowoki, S. Shinjiro, T. Ando and T. Hanafusa, *Chem. Lett.*, 1983, 379.

153. K. S. Suslick, J. W. Goodale, H. H. Wang and P. F. Schubert, *J. Am. Chem. Soc.*, **105** (1983), 5871.

154. C. Petrier and J. L. Luche, *Tetrahedron Lett.*, **28** (1987), 2347.

155. P. Boudjouk, *Organometallics*, **5** (1986), 1257.

156. A. S. Szalay, *Phys. Chem.* A., **164** (1933), 234.

157. A. S .Gyorgi, *Nature*, **131** (1933), 278.

158. E. W. Flosdorf and L. A. Chambers, *J. Amer. Chem. Soc.*, **55** (1933), 3051.

159. E. Chang, P. Hamet and J. Tremblay, *J. Cardiov. Pharmac.*, **23** (1994), S42.

160. W. C. Koskinen, K. E. Sellung, J. M. Baker, B. L. Barber and R. H. Dowdy, *J. Environ. Sci. Health*, **29** (1994), 581.

161. S. Mamba and B. Kratochvil, paper presented at 24-th International Symposium on Environmental Analytical Chemistry, 15-19 May 1994, Ottawa, Canada.

162. C. B. Boss and K. J. Fredeen, "Concepts, Instrumentation and Techniques in ICP-OES", The Perkin Elmer Corporation, Norwalk, CT, 1989.

163. P. W. J. M. Boumans and F. J. deBoer, *Spectrochim. Acta.*, **30B** (1975), 309.

164. V. A. Fassel and B. R. Bear, *Spectrochim. Acta.*, **41B** (1986), 1089.

165. J. Anderson, *At. Spectrosc.*, **13** (1992), 99.

166. Q. Jin, C. Zhu, K. Brushwyler and G. Hieftje, *App. Spectrosc.*, **44** (1990), 183.

167. A. P. Edwards and J. M. Bremer, *J. Soil Sci.*, **18** (1967), 47.

168. N. Miller-Ihli, *J. Anal. At. Spectrom.*, **3** (1988), 73.

169. N. Miller-Ihli, *Fresenius J. Anal. Chem.*, **337** (1990), 271.

170. M. S. Epstein, G. R. Carnick, W. Slavin and N. Miller-Ihli, *Anal. Chem.*, **61** (1989), 1414.

171. A. H. van den Akker and H. van den Heuvel, *At. Spectrosc.*, **13** (1992), 72.

172. K. S. Suslick, *Science*, **247** (1990), 1439.

173. D. M. Kumina, A. V. Karykin and I. F. Gribovskaya, *Russ. J. Anal. Chem.*, Engl. Trans., **40** (1985), 930.

174. A. V. Karyakin, M. S. Pomares Alfonso, D. M. Kumina, Y. I. Belyaev and N. P. Starshinova, *Russ. J. Anal. Chem.*, Engl. Trans., **44** (1990), 1201.

175. A. A. Hinds and L. E. Lowe, *Can. J. Soil Sci.*, **60** (1980), 329.

176. M. Escudey, M. de la Luz Mora, P. Diaz and G. Galindo, *Clays and Clay Minerals*, **37** (1989), 493.

177. M. Akcay and S. Savasci, *Analyst*, **114** (1989), 1079.

178. M. I. Saleh, M. S. Jab, I. Ab Rahman and S. Norasia, *Analyst*, **116** (1991), 743.

179. L. Rayleigh, *Philos. Mag.*, **34** (1917), 94.

180. F. D. Smith, *Philos. Mag.*, **19** (1935), 1147.

181. L. A. Crum, *J. Acoust. Soc. Am.*, **68** (1980), 203.

182. E. A. Neppiras, *Phys. Rep.*, **61** (1980), 159.

183. A. Hanglein, *Ultrasonics*, **25** (1987), 6.

184. A. L. Loomis, *Phil. Mag.*, vii, **4** (1927), 417.

185. S. Mamba and B. Kratochvil, *J. Int. Environ. Anal. Chem.*, in press.

186. B. Pugin, *Ultrasonics*, **25** (1987), 49.

187. B. Kratochvil and W. Harris, "An Introduction to Chemical Analysis", Sanders College Publishing, Philadelphia, 1981.

188. T. J. Mason, *Canadian Chemical News*, March 1991, 25.

189. W. Lauterborn and W. Hentschel, *Ultrasonics*, **24** (1986), 59.

190. W. Lauterborn, "Cavitation and Inhomogeneities in Underwater Acoustics", Springler-Verlag, New York, 1980.

191. W. Lauterborn and H. Bolle, *J. Fluid Mech.*, **72** (1975), 391.

192. A. Mills, H. L. Davies and M. S. Garley, *Chem. Soc. Faraday Trans.*, **86** (1990), 2163.

193. W. T. Richards and A. L. Loomis, *J. Am. Chem. Soc.*, **49** (1927), 3086.

194. P. Chabrecek, L. Soltes, Z. Kallay and I. Novak, *Chromatographia*, **30** (1990), 201.

195. W. Burnaby, *Proc. Inst. Civ. Eng.*, **122** (1895), 67.

196. K. S. Suslick, D. A. Hammerton and R. E. Cline, Jr., *J. Am. Chem. Soc.*, **108** (1986), 5641.

197. E. B. Flint and K. S. Suslick, *Science*, **253** (1991), 1397.

198. K. Makino, M. Mossoba and P. Riesz, *J. Phys. Chem.*, **87** (1983), 1369.

199. F. O. Schmitt, C. H. Johnson and A. R. Olson, *J. Am. Chem. Soc.*, **51** (1929), 370.

200. M. E. Fitzgerald, V. Griffing and J. Sullivan, *J. Chem. Phys.*, **25** (1956), 926.

201. Instrumental Analysis (CHEM 313) Laboratory Manual, "Experiment 6: Polarography" Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada.

202. F. E. Fox and G. D. Rock, *J. Acoust. Soc. Am.*, **12** (1941), 505.

203. T. J. Mason and J. P. Lorimer, "Sonochemistry: Theory, Aplications and uses of Ultrasound in Chemistry", pages 51-53. John Wiley and Sons, New York, 1988.

204. T. J. Mason and J. P. Lorimer, "Sonochemistry: Theory, Aplications and uses of Ultrasound in Chemistry", pages 58-60. John Wiley and Sons, New York, 1988.

205. T. J. Mason and J. P. Lorimer, "Sonochemistry: Theory, Aplications and uses of Ultrasound in Chemistry", page 48. John Wiley and Sons, New York, 1988.

206. M. Hitchman, "Measurement of Dissolved Oxygen", John Wiley and Sons, New York, 1978.

207. L. Winkler, *Ber. Deut. Chem. Res.*, **21** (1888), 2843.

208. L. C. Clark, *J. Appl. Physiol.*, **6** (1953), 189.

209. I. Kolthoff and R. Miller, *J. Am. Chem. Soc.*, **63** (1941), 1013.

210. E. Moore, *Sewage Works J.*, **20** (1948), 1041.

211. G. Cum, G. Galli, and R. Gallo, *Ultrasonics*, **30** (1992), 267.

212. J. Jacq and O. Bloch, *Electrochim. Acta.*, **15** (1970), 1945.

213. R. Cai, K. Hashimoto and A. Fujishima, *J. Electroanal. Chem.*, **326** (1992), 345.

214. A. Kotronau and F. Hoffman, *J. Phys. Chem.*, **95** (1991), 3630.

215. J. K. Koch, "Free Radicals", vol. 2, John Wiley, New York, 1973.

216. B. Kratochvil and W. Harris, "An Introduction to Chemical Analysis", Sanders College Publishing, Philadelphia, 1981.

217. J. E. Falk and J. N. Phillips, "Chelating Agents and Metal Chelates", F. P. Dwyer and D. P. Mellor (eds.), Academic Press, New York, 1964, 452.

218. R. Surtane and H. F. Steger, *Talanta*, **32** (1985), 439.

219. S.A. Abboud, "Proceedings of the 4th Annual WEALA Workshop on Heavy Metals in the Environment", p2-1, Alberta Research Council, Edmonton, Alberta, 1991.

220. C. Petrier, M. Micolle, G. Merlin, J. L. Luche and G. Reverdy, *Environ. Sci. Technol.*, **26** (1992), 1639.

221. C. Huang and E. Schulte, *Commun. Soil Sci. Plant Anal.*, **16** (1985), 943.

222. B. Kratochvil, N. Motkosky, M. J. M. Duke and D. Ng, *Can. J. Chem.*, **65** (1987), 1047.

223. F. A. Cotton and G. Wilkinson, "Advanced Inorganic Chemistry", 2nd ed., Wiley, New York, 1966. p804.

224. K. Okamoto, Certificate of Analysis of Reference Material #6, Mussel, National Institute for Environmental Studies, Ibaraki, Japan, 1983.

225. S. D. Rasberry, Certificate of Analysis of Standard Reference Material 1575, Pine Needles. National Bureau of Standards, Gaithersburg, MD, 1982.

226. K. Okamoto, Certificate of Analysis of Reference Material #10c, Rice Flour-Unpolished, National Institute for Environmental Studies, Ibaraki, Japan, 1987.

227. E. S. Gladney, C. E. Burns, D. R. Perin, I. Roelandts and T. E. Gills, 1982 Compilation for Elemental Concentration Data for NBS Biological, Geological and Environmental Standard Reference Materials. US Department of Commerce, Gaithersburg, MD. page 8.

228. M. Ihnat, Report of Analysis of Reference Material 8412, Corn (*Zea mays*) Stalk. Agriculture Canada, Ottawa, Ontario, 1981.

229. E. S. Gladney, C. E. Burns, D. R. Perin, I. Roelandts and T. E. Gills, 1982 Compilation for Elemental Concentration Data for NBS Biological, Geological and Environmental Standard Reference Materials. US Department of Commerce, Gaithersburg, MD. page 8.

230. M. Ihnat, Report of Analysis of Reference Material 8435, Whole Milk Powder. Agriculture Canada, Ottawa, Ontario, 1981.

231. S. D. Rasberry, Certificate of Analysis of Standard Reference Material 1577a, Bovine Liver. National Bureau of Standards, Gaithersburg, MD, 1985.

232. K. Okamoto, Certificate of Analysis of Reference Material #5, Human Hair, National Institute for Environmental Studies, Ibaraki, Japan, 1985.

233. R. Q. Thompson and M. Ghadiali, *J. Chem. Ed.*, **70** (1993), 170.

234. B. Carlin, "Ultrasonics", Chapter 4. McGraw Hill, New York, 1960.

235. G. J. Price, "Current Trends in Sonochemistry", page 144, Royal Society of Chemistry, Cambridge, 1992.

236. Titanium Metal Products Data Sheet, Elecronic Space Products International, Aguora Hills, CA.

237. D. Ensminger, "Ultrasonics", Chapter 4. Marcel Dekker Inc., New York, 1973.

238. P. Smith, *Harz. Mat. Manag.*, **4** (1992), 37.

239. K. W. Pratt, H. M. Kingston, W. A. MacCrehan and W. F. Koch, *Anal. Chem.*, **60** (1988), 2024.

240. B. D. Zehr, J. P. VanKuren and H. M. McMahon, *Anal. Chem.*, **66** (1994), 2194.

241. Ace Glass Model 8910 Ultrasonics Processor Operating Instructions Manual, Ace Glass Inc., Vineland, NJ. 1990.